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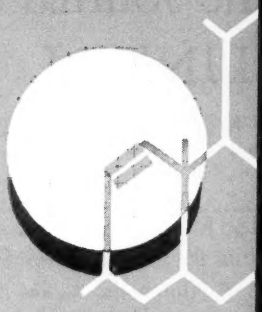

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HYPOPHYSECTOMY IN MAN: EXPERIENCES IN SEVERE DIABETES MELLITUS*

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THE significance of the hypophysis in experimental diabetes mellitus is well established. At least two of the pituitary hormones exert a diabetogenic action, namely, the adrenocorticotropic hormone and the growth hormone. The early experiments by Houssay *et al.* (1) demonstrated that hypophysectomy produces a marked amelioration of diabetic symptoms in the depancreatized animal and in the animal made diabetic by alloxan.

Clinical observations have shown that also in humans pituitary insufficiency may ameliorate the diabetic state (2–6). In 1936 Chabanier *et al.* (7) reported on hypophysectomy in a case of severe diabetes mellitus. After removal of the pituitary gland the diabetes could be reasonably well controlled. There was no autopsy, but from the fact that postoperatively the patient required as much as 70–80 units of insulin per day, we are inclined to believe that functioning pituitary tissue was still present.

A case described by Poulson (8) is of special interest. In a 30-year-old woman with diabetes for twenty years and with diabetic retinopathy, Sheehan's syndrome developed. Her insulin sensitivity increased and the retinopathy disappeared, showing that diabetic vascular complications may also be influenced by pituitary insufficiency.

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The introduction of insulin into the treatment of diabetes mellitus has increased the life expectancy of juvenile diabetics. On the other hand, the longer survival has been accompanied by an increasing incidence of diabetic vascular complications. For these, no specific treatment is known. The present paper reports on the effect of hypophysectomy in longstanding juvenile diabetes mellitus with progressive malignant vascular complications. A preliminary report on Case 1 was given in 1952 (9). Only patients in whom either a fatal outcome or blindness was inevitable were selected for this procedure initially.

CASE REPORTS

Initially the series consisted of 7 patients, of whom 3 are living from one to two and a half years after the operation (Cases 1, 5, and 6). In addition 2 patients are reported who were operated upon two months ago.

Case 1. B.M. was a married man, born in 1921. Diabetes mellitus was diagnosed in *1934*. He had received insulin since *1936* and a free diet. The daily insulin dose had averaged between 50–60 units of protamine-zinc-insulin. The first complications were observed in *July 1950*—extensive bilateral retinal hemorrhages and exudates and an albuminuria of 0.2 per cent. The patient also showed rather extensive edema of the eyelids and ankles. The serum nonprotein-nitrogen level was 31–42 mg. per 100 ml., hemoglobin 67 per cent, and red blood cell count 3.8 million per cu. mm. The resting blood pressure ranged from 165/105 to 140/70 mm. of Hg. Creatinine clearance was 152 ml. per minute. The patellar and ankle jerks could not be elicited. Vision deteriorated rapidly. In *January 1951* only light perception remained. The albuminuria persisted, and red cells appeared in the urinary sediment. There was occasional edema of the ankles. The range of blood pressure readings was between 150/90 and 180/110. Creatinine clearance had decreased to 76 ml. per minute. Since *September 1951* there had been total amaurosis. During the last months there was a marked tendency to anemia, which was treated by blood transfusions.

Preoperative examination in *November 1951:* The patient was pale and had slight edema of the ankles and eyelids. The resting blood pressure ranged between 175/80 and 190/110; occasionally a normal resting blood pressure was noted. Body weight was 70 Kg. and body length, 181 cm. Hemoglobin concentration was 63 per cent and the red cell count was 3.5 million. There was no ketonuria. The fasting blood sugar level varied between 150 and 400 mg. per 100 ml. The daily excretion of sugar in the urine was about 5 to 35 grams. There was an albuminuria of 0.2 per cent, and occasionally red cells and hyaline casts were seen in the urine. The serum nonprotein-nitrogen level was 30–40 mg. per 100 ml. Creatinine clearance was 75 ml. per minute. Eye examination showed large hemorrhages, exudates and proliferative changes in both eyes, and complete amaurosis. Roentgenograms of the extremities showed slight calcification of the large veins but not of the arteries.

The daily insulin requirement was 40 units of protamine-zinc, and 20 units of regular insulin.

Summary. A 30-year-old male suffering from diabetes mellitus for seventeen years, showed very rapidly progressing diabetic retinopathy, resulting in amaurosis one year after its diagnosis; there was progressive nephropathy, with constant albuminuria and

edema, moderate elevation of the blood pressure and peripheral vascular calcification.

Hypophysectomy was performed on November 30, 1951. The patient was not given any hormone substitution therapy except for insulin before, during, and for the first two months after the operation. The postoperative course was uncomplicated, and he left the hospital one month after the operation. At this time he required 12 units of regular insulin per day. (Final hormone substitution therapy and later course are described in subsequent sections.)

Case 2. K.E.S.A. was a married man, born in 1923. He had had diabetes mellitus since the age of 11 and had taken insulin since *1936.* His insulin requirement was about 70 units of protamine-zinc-insulin while on a free diet. Very rapidly progressing diabetic retinopathy had been noted since *1951,* leading to amaurosis of the left eye and a vision of 0.7[1] in the right eye at the time of hypophysectomy. Extensive exudates and hemorrhages were then observed in the eyegrounds. Albuminuria had been observed since *1951.* Resting blood pressure ranged between 140/100 and 170/110 mm. of Hg. Kidney function was well preserved, with an inulin clearance of 108 and a PAH-clearance of 565 ml. per minute.

Hypophysectomy was performed on May 5, 1952. The operation was complicated by a severe hemorrhage from the sella, which was difficult to check. The patient did not wake up from the anesthesia. In the evening of the day of operation the systolic blood pressure fell to values below 100 mm. of Hg, and the pulse was irregular and soft. There was no acidosis, and the blood sugar level remained at about 200 mg. per 100 ml. The patient was given whole blood, physiologic saline, cortisone (100 mg. before the operation and 100 mg. afterwards), Eschatin (50 ml. intravenously and 10 ml. every two hours), circulatory stimulants and penicillin. As an intracranial hemorrhage could not be excluded, re-operation was undertaken the same evening. No hematoma was found. The brain showed a moderate edema, which necessitated the resection of a small part of one frontal pole. Despite intense stimulation therapy, the patient expired on May 6. Autopsy revealed that the hypophysectomy had not been complete.

Case 3. I.M.H. was a single woman, born in 1928, who had had diabetes mellitus since *1937.* Insulin had been started the same year. Her daily insulin requirement was about 50 units of protamine-zinc-insulin. Albuminuria had been present since *1949,* and turbidity of the lenses and retinal exudates and hemorrhages since *1951.* During the few months before she was seen by us, there had been very rapidly progressing retinal changes, leading to a vision of 0.2–0.3 in both eyes. The glomerular filtration rate was 81, and the renal plasma flow 319 ml. per minute. Albuminuria measured 0.2 per cent. The resting blood pressure was 150–160/100–110 mm. of Hg. The serum nonprotein-nitrogen level was 40–60 mg. per 100 ml., hemoglobin 69 per cent, and red blood cell count 3.6 million per cu. mm. A moderate tendency to edema was noted.

Hypophysectomy was performed on June 10, 1952. The immediate postoperative course was uneventful. However, it was soon observed that antibiotics had to be given continuously; a rise in temperature followed every attempt to discontinue this treatment. The cause could not be demonstrated. On July 7 the patient reported an insulin reaction of moderate degree. On July 11 she became comatose but woke up after intravenous infusion of a small amount of 30 per cent glucose. In the evening of the same day she again became comatose, with frequent epileptic fits, falling blood pressure, superficial breathing and soft pulse. The blood sugar level at this time was 240 mg. per 100 ml. An

[1] Monoyer's system (normal vision, 1.0).

infusion of 60 ml. of 30 per cent glucose was without effect. The patient was also given ACTH and Eschatin intravenously. An epileptic state developed and the patient expired the next day. Autopsy did not reveal the immediate cause of death. Serial sectioning of the sella showed a complete hypophysectomy.

Case 4. M.L.M. was a married woman, born in 1923 (grav. IV, para II). Diabetes mellitus had been discovered in *1915*, and she had been immediately started on insulin (60 units daily) and a free diet. Extensive retinal exudates and hemorrhages developed toward the end of *1948*. The right eye became amaurotic in the summer of *1949*. Rapidly progressing disturbance of vision of the left eye was obvious in the fall of *1950;* a cataract developed, and the eye became almost amaurotic in *1952*.

In *January 1953* the creatinine clearance was 60 ml. per minute, and the PAH-clearance 216 ml. per minute. The resting blood pressure was 150/105 mm. of Hg. There was no peripheral vascular calcification. The level of serum nonprotein nitrogen was 38–50 mg. per 100 ml. There were bilateral cataracts, with amaurosis of the right eye, but perception of light in the left eye. Electroretinograms showed no response in either eye. The blood hemoglobin level was 60 per cent. Albuminuria was 0.2 per cent per 24-hour specimen. There was moderate edema of the eyelids and ankles. The total plasma protein level was 6.2–6.6 Gm. per cent, with 3.9 Gm. per cent of albumin. The daily insulin requirement had decreased during the previous four years from about 70 units in 1948 to about 40 units in 1953.

Summary. A 30-year-old woman with diabetes since the age of 12, had very rapidly progressing retinal changes since 1948 (leading to almost complete blindness in 1952), nephropathy of unknown duration with constant albuminuria and moderate elevation of blood pressure, and a marked tendency to anemia and edema.

Hypophysectomy was performed on March 23, 1953. The immediate postoperative course was complicated by frequent epileptic fits on March 30 and 31. Re-operation was undertaken on April 1, and a very small epidural hematoma was removed. The epileptic fits did not reappear after this operation. The insulin dose had to be reduced gradually after the third postoperative week. The patient left the hospital on April 26, taking 8 units of protamine-zinc-insulin per day and, every second to third week, 25 mg. of desoxycorticosterone trimethylacetate and 50 Gm. of testosterone isobutyrate in microcrystalline suspension. The blood pressure as well as the serum nonprotein nitrogen remained at a normal level. The anemia was treated by transfusions of whole blood at intervals of approximately one month. Except for one attack of severe hypoglycemia, treated as reported subsequently, the course was rather uneventful. For ten months after the hypophysectomy the patient was able to take care of her apartment and her two children with the aid of a maid. Now and then she had periods of anorexia, with a tendency to weight loss and increased fatigue. Following the implantation of 200 mg. of desoxycorticosterone acetate (DCA), hypertension developed, with circulatory failure and an increase in the level of serum nonprotein nitrogen. Although the renal and cardiac failure persisted, the patient noted a gradual increased sense of well-being. After two months of hospitalization, she was discharged on June 12. The concentration of NPN was then 70 mg. per 100 ml. and the blood pressure, 160/120 mm. of Hg. On June 22 she became comatose, and had epileptic fits of Jacksonian type on the left side of the body. The blood sugar level was about 300 mg. per 100 ml., the NPN 73 mg. per 100 ml., and the blood pressure 230/130. The patient expired the next morning. At autopsy no obvious lesions in the brain could be seen on macroscopic examination, except for a swelling of the right frontal lobe and two fresh blood clots in this area.

Case 5. A.M.H. was a single woman, born in 1926. Diabetes mellitus had developed in *1939*. She had immediately been started on insulin, about 50 units per day. Albuminuria was diagnosed in *1946*, and it was observed that the retinal arteries and veins were tortuous. In *1948* she was treated in the hospital for pyelonephritis. Retinal changes were discovered one year later. In *1950* it was observed that her vision had deteriorated markedly. The albuminuria was very heavy at this time, but the urinary sediment was normal. The blood pressure was 120/80 mm. of Hg. An intravenous pyelogram showed normal findings. In both eyes severe proliferative changes were seen. In the right eye there were exudates and hemorrhages. The level of serum nonprotein nitrogen was 64 mg. per 100 ml. The glomerular filtration rate was 42, and the renal plasma flow, 285 ml./per min. During *1951* the vision decreased bilaterally to 0.2. Ulcers of the feet developed, and these healed with difficulty. In *1952* she had extensive edema of the face and lower extremities. At this time the vision was less than 0.1 bilaterally, and very advanced proliferative retinal changes were seen. The fasting blood sugar level was 200–300 mg. per 100 ml., and she excreted 5–40 grams of sugar daily in the urine while on a free diet.

During *1953* her general condition deteriorated, and she had constant headaches, anorexia and fatigue. The preoperative examination in *April 1953* showed a pale patient with marked edema of the face and lower extremities. Resting blood pressure was 145/90. She showed decreased pain, tactile and temperature sensitivity and decreased vibratory sensitivity of both feet. Ankle and patellar jerks were very weak. The hemoglobin level was 55 per cent; red blood cell count 3.3 million; serum NPN level 39–50 mg. per 100 ml; albuminuria 0.1–0.3 per cent ; rare red and white cells in the urinary sediment; and total plasma protein level 6.4 Gm. per cent, with albumin 3.2 Gm. per cent. There was no calcification of the peripheral vessels. Creatinine clearance was 57 ml./min., insulin clearance 42 ml./min., and PAH-clearance 261 ml./min. Fasting blood sugar concentration was 200–800 mg. per 100 ml.; urinary sugar excretion was 5–50 Gm. daily; but there was no ketonuria. The daily insulin requirement had decreased to 24 units. Examination of the eyes showed the vision of the right eye to be 0.5/60; with the left eye, only movement could be distinguished; in the eye grounds very advanced proliferative changes were noted; electroretinograms of both retinae showed no response.

Summary. A 26-year-old-woman with diabetes mellitus since the age of 13, had nephropathy since 1946, retinal changes since 1949, ulcers of the feet since 1951; and rapidly progressing retinal and renal lesions leading to almost complete blindness and a nephrotic syndrome in 1952.

Hypophysectomy was performed on April 29, 1953. The immediate postoperative course was complicated by a urinary infection, which responded well to antibiotics. The main obstacle for several months after the operation was periods of anorexia and vomiting, which made hormone substitution therapy difficult. For short periods of time she had to be fed by a tube. The anorexia was followed by a further decrease in the concentration of plasma protein, which gave rise to general edema. Because of this and the marked tendency to anemia, transfusions of whole blood were given at frequent intervals. Within a couple of months after the operation it was possible to omit insulin. It was later found that the anorexia to a marked extent was due to psychic factors, mainly the patient's anxiety about leaving the hospital. When suitable arrangements had been made, her general condition improved slowly, and she was discharged in fairly good condition in May 1954. The blood pressure was then normal and so was the level of serum nonprotein nitrogen. She had no edema. She has continued to show improvement and has required no insulin while on a free diet.

Case 6. B.L.E., a single woman, was born in 1925. Diabetes mellitus was diagnosed in *1934*. The daily insulin requirement ranged between 40 and 60 units while on a free diet. In *1942* she had a fasting blood sugar level of 300–400 mg. per 100 ml. She had ketonuria but had not been comatose; there was no albuminuria and no edema. Resting blood pressure was 140/80 mm. of Hg. Body weight was 49 Kg. and body length was 161 cm.

On examination in *November 1951* the patient was pale and looked edematous. The resting blood pressure was 160/100. She had 0.1 per cent of albumin in the urine, and the level of serum nonprotein nitrogen was 36 mg. per 100 ml. Eye examination showed vision to be 0.5–0.6 bilaterally; there were advanced retinal changes with hemorrhages and exudates, large tortuous veins and widened capillaries, but no proliferative changes.

On examination in June *1951* the eye changes had progressed; the vision was 0.1 in the right eye and 0.6 in the left and there were proliferative changes in the right eye-ground. Resting blood pressure was 170/105. The albuminuria remained unchanged.

In *April 1953* the patient appeared pale and puffy. Resting blood pressure was 145/100. Body weight was 51 Kg. and body length, 163 cm. The hemoglobin level was 67 per cent and the red cell count 3.6 million. There was no ketonuria. About 10–60 Gm. of sugar and 0.1 per cent of albumin were excreted in the urine per day; there were no pathologic findings in the sediment. The fasting blood sugar level was 200–500 mg. per 100 ml., total plasma protein 7.1 Gm. per cent (with 5.2 Gm. per cent albumin), and NPN 37 mg. per 100 ml. Inulin clearance was 76 ml./min., and PAH clearance 339 ml./min. Eye examination showed vision in the right eye to be 0.1, and in the left eye, 0.6. There was slight peripheral turbidity of the lenses, bilateral hemorrhages in the eyegrounds, and a large proliferative streak above the right papilla.

Summary. A 28-year-old woman with diabetes mellitus for nineteen years, had rapidly progressing diabetic retinopathy, moderate nephropathy with constant albuminuria, and slight elevation of the blood pressure and edema.

Hypophysectomy was performed on May 15, 1953. The postoperative course was complicated by a severe pyelonephritis, which was treated by antibiotics for several months, and which made it necessary to keep the cortisone dosage at a level of about 25–50 mg. per day. When an attempt was made to lower the dose of cortisone without simultaneous lowering of the dose of insulin, there followed a severe attack of hypoglycemia. Administration of DCA in addition to the dose implanted (see Table 3) was followed by acute retinal edema and a moderate general edema. These changes disappeared after the DCA was withdrawn and the patient was kept on a low-salt diet for a week. When the patient left the hospital in March 1954 she was in a very good general condition with a normal blood pressure and nonprotein nitrogen level. The albuminuria had disappeared. The vision had not decreased further.

Case 7. A.R.G.S., a single male, born in 1930 had had diabetes mellitus since the age of 3. He had been treated with insulin from the start of the disease. The daily insulin requirement increased from 14 units in *1933* to 48 units in *1935*. Albuminuria was observed for the first time in *1948*. At this time the resting blood pressure was 120/95 mm. of Hg. The daily urinary excretion of sugar was 20–80 Gm. In *1949* the blood pressure was 160/110 at one examination; the patient also showed edema of the eyelids. He excreted 30–75 Gm. of sugar per day while on a free diet plus 56 units of insulin. In *1951* there was constant albuminuria of 0.1 per cent, several red cells in the sediment, and a resting blood pressure of 160/115. The fasting blood sugar level was 350–600 mg. per 100 ml. and the insulin requirement was 64 units. There was frequent epistaxis. The vision of the right eye was 0.7, and of the left eye, 0.9; hemorrhagic spots were seen in both eye-grounds.

In *December 1952* there was deterioration, with marked edema of the face and lower limbs. Blood pressure was 180/120 and the serum NPN level was 40–50 mg. per 100 ml. The vision of the right eye was 0.2, of the left eye 0.5, and there were progressing exudates and hemorrhages in the eyegrounds. The condition grew worse, and at the preoperative examination in *October 1953* increasing fatigue and impairment of vision were noted. Edema and headaches appeared frequently, as well as paresthesias in both legs. The patient was pale and had slight edema of the face. Resting blood pressure was 220/110. Ankle and patellar jerks could not be elicited, and there was decreased vibration sensitivity in the feet. Hemoglobin was 68 per cent and the red blood cell count 3.5 million. The fasting blood sugar level was 400–600 mg. per 100 ml.; urinary sugar was 40–70 Gm. per day on a daily insulin dose of 88 units and a free diet; and there was no ketonuria. There was 0.2–0.4 per cent albumin in the urine, and red cells in the sediment. The serum albumin level was 3.6 Gm., globulin 2.3 Gm. per cent, and NPN 40 mg. per 100 ml. Advanced calcification of the large vessels of the legs and arms was evident on x-ray examination. Inulin clearance was 68 and PAH clearance was 453 ml. per minute. The vision of the right eye had decreased to perception of hand movements and was 2/60 on the left. Advanced hemorrhages and proliferative changes were noted in both eyegrounds, as well as a fresh large preretinal hemorrhage in the right eye.

Summary. A 23-year-old man suffering from diabetes since the age of 3, had progressing renal lesions since 1948 and retinal changes since 1951, leading to almost total blindness within two years. There was constant albuminuria, edema and hypertension, and advanced calcifications of the peripheral vessels.

Hypophysectomy was performed on December 3, 1953. The operation was complicated by continuous bleeding from the intracranial blood vessels, which were very brittle. This necessitated removal of one frontal pole of the brain. The postoperative course was complicated by bouts of epileptic fits, which were influenced favorably by cortisone. For some weeks the patient seemed to recover normally. However, it was soon observed that he was deteriorating mentally despite the fact that the diabetic state, as such, could be handled in a manner similar to that in the other cases. The patient finally reached a state of stupor and expired on May 30, 1954. The autopsy revealed advanced general arteriosclerosis.

Addendum

Two further patients with severe diabetes mellitus were recently hypophysectomized. Because of the short time of observation, only a brief description of these cases is given. They are not included in the tables nor in the discussion.

Case 8. K.E.B., a married male, was born in 1923. He had had diabetes mellitus since *1935*, and had been treated with insulin since *1937*. Since *1952* he had observed increased tiredness and a decreased mental alertness. Now and then he had short fits of petit mal. In July 1953 the vision decreased acutely, and this condition persisted. Albuminuria was observed in 1952, and in 1953 the systolic blood pressure was 160–170 mm. of Hg. At the preoperative examination in May *1954* the resting blood pressure was 210/100, the level of serum nonprotein nitrogen was 41 mg. per 100 ml., there was an albuminuria of 0.1–0.2 per cent, and the sediment showed several white and red cells per visual field. The glomerular filtration rate was 65–84 and effective renal plasma flow was 409–550 ml. per min. The vision of the right eye was 0.7 and of the left eye 0.5; in the eyegrounds a moderate amount of hemorrhage and slight exudates were seen.

The patient was *hypophysectomized on May 20, 1954.* During the operation a super-

ficial lesion the size of a pea was made on the right frontal pole. On the day of operation he was given 150 mg. of cortisone, and the dose was reduced successively after the operation. The postoperative course was complicated by episodes of epileptic fits. DCA was not given in this case, and the present daily hormone substitution therapy two months after the operation consists of 20 mg. of cortisone, 12 units of protamine-zinc-insulin and thyroid hormone equivalent to 90 micrograms of thyroxine.

Case 9. G.E., single woman, was born in 1932, and had had diabetes mellitus since *1941*. During the period 1941–54 she had had several episodes of diabetic and of hypoglycemic coma, and the insulin dose frequently had to be altered. A decrease in vision was noticed for the first time in *1952*, and this gradually progressed. At the same time albumin appeared in the urine, and a blood pressure of 160/100 mm. of Hg. was noticed. For one month prior to operation the patient had several attacks of diabetic and of hypoglycemic coma. The final preoperative insulin dosage was 72 units of regular insulin divided into three doses per day. Her resting blood pressure was 215/110, the level of serum nonprotein nitrogen was 44 mg. per 100 ml., there was an albuminuria of 0.1–0.2 per cent, and the sediment showed a single red cell per visual field. She had moderate general edema. The glomerular filtration rate was 44, and the effective renal plasma flow was 246 ml. per minute. The preoperative period was complicated by a severe secondary glaucoma in both eyes, which had almost disappeared at the time of operation. The vision in the right eye was 0.1, but in the left eye there was only slight perception of light.

The patient was *hypophysectomized on June 6, 1954*. The postoperative course was uncomplicated, and at present, two months after the operation, she is up and about, has a blood pressure of 130/90 and a normal NPN level. Vision is unchanged. The albuminuria has disappeared. She was given 50 mg. of cortisone on the day of operation. Her present daily hormone substitution therapy consists of 15 mg. of cortisone and 8 units of protamine-zinc-insulin. The diabetic state is stable with this single dose of insulin. The fasting blood sugar level is 200–300 mg. per 100 ml., and urinary sugar excretion is 0–10 Gm. per day. As in the preceding case, treatment with DCA was avoided.

COMMENT

Table 1 shows the ages of the patients at the onset of the diabetes, at the time of diagnosis of retinal and renal changes, and at the time of hypophysectomy.

In Table 2 a summary is given of some important clinical data in Cases 1, 4, 5, 6 and 7 concerning the status of the eyes, kidneys and cardiovascular system at the time of hypophysectomy. It is obvious from this table as well as from the case histories that all patients had progressing impairment of renal function and diabetic retinopathy. There was almost total amaurosis in all cases except No. 6. The impairment of renal function amounted to at least 50 per cent according to the clearance values in Cases 1, 4 and 5; in Cases 6 and 7 the impairment of renal function was moderate. On the other hand, Case 7 showed the highest blood pressure in this series as well as very extensive vascular calcification. The patient in Case 5 had a nephrotic syndrome, whereas the remaining patients showed puffiness of the face.

TABLE 1. DATA REGARDING THE DEVELOPMENT OF THE DISEASE

Case No.	Sex	Born	Age at onset of diabetes (yrs.)	Age at diagnosis of retinal changes (yrs.)	Age at diagnosis of renal disease (yrs.)	Date of hypophysectomy	Age at hypophysectomy (yrs.)
1	M	1921	13	29	29	Nov. 1951	30
2	M	1923	11	28	28	May 1952	29
3	F	1928	9	23	12	June 1952	24
4	F	1923	12	25	?	Mar. 1953	30
5	F	1926	13	23	20	Apr. 1953	27
6	F	1925	9	26	26	May 1953	28
7	M	1930	3	21	18	Dec. 1953	23

TABLE 2. CLINICAL DATA AT THE TIME OF HYPOPHYSECTOMY

Case No.	Eyes						Kidneys			
	Vision		Lens turbidity	Retinal changes			Albuminuria (%)	Serum NPN (mg./100 ml.)	GFR (ml./min.)	RPF (ml./min.)
				Exudation	Hemorrhage	Proliferation				
1	Amaurosis	O.D.	−	+++	+++	+++				
	Amaurosis	O.S.	−	+++	+++	+++	2	30–40	75*	
4	Amaurosis	O.D.	+++	?	?	?				
	Percept. of light	O.S.	++	+++	+++	+++	2	38–50	60*	216
5	Vision 0.5/60	O.D.	+	−	−	+++				
	Near amaurosis	O.S.	+	−	−	+++	1–3	38–50	42 57*	261
6	Vision ≦0.1†	O.D.	+	+	++	+				
	Vision ≦0.6	O.S.	+	+	++	−	1	37	87	386
7	Near amaurosis	O.D.	−	+	+++	++?				
	Vision 2/60	O.S.	−	+	+++	++?	2–4	40	68	453

Case No.	Cardiovascular			Blood				
	Blood pressure (mm. Hg)	Heart size (ml./M²)	Calcific. of large vessels (x-ray)	Serum albumin (Gm. %)	Serum globulin (Gm. %)	Hgb. %	Red cells million/cu. mm.	Edema
1	175/80–190/110	310	+			63	3.5	+
4	150/105	330	−	3.9	2.7	60	2.8	+
5	145/100–170/105	360	−	3.2	3.2	55	3.3	+++
6	145/100		−	5.2	1.9	67	3.6	+
7	220/110	420	+++	3.6	2.3	68	3.5	+

* Creatinine clearance.

† Monoyer's system (normal vision, 1.0).

Preoperative care

The preoperative care of the patients included correction of the anemia with transfusions of whole blood. The electrolyte and fluid balances were controlled, and edema was treated according to current methods. All patients were kept on their usual insulin dose. None of them had ketonuria preoperatively.

In Cases 3–7, various dosages of DCA, of cortisone, or both were administered during the week before operation.

Medical treatment during *the day of operation* included the administration of insulin and cortisone and the administration of blood and fluids, as well as the control of the diabetic state. Penicillin was always started the day of operation and continued for about one week.

In all cases, half of the usual daily insulin dose was given as regular insulin in the morning on the day of operation, which was performed two to three hours later. In none of the cases was it necessary to add more insulin during this first day. No acidosis appeared, and the blood sugar was usually at a level of between 300–600 mg. per 100 ml.

Except in Case 1, cortisone in a dose of 100 mg. was given intramuscularly before and immediately after the operation. In addition 50 ml. of Eschatin was given intravenously toward the end of the operation, followed by 10 ml. every two hours.

Postoperative period

Insulin was given during the few days immediately after operation in the dose started on the day of operation. If necessary, regular insulin was added in doses of 4–16 units. After about one week the daily dose was given as protamine-zinc-insulin. When the dose of cortisone was decreased and finally discontinued, the dose of insulin had to be decreased correspondingly. In general, when the patients were no longer receiving cortisone, the daily insulin requirement was about 8 units of protamine-zinc-insulin.

Glucose in physiologic saline was continued intravenously until the second or third day, when the patients generally were able to drink freely. They were then kept on an ordinary hospital diet with a reduced amount of sugar. Acidosis did not appear postoperatively, but traces of ketone bodies appeared in the urine during the administration of larger amounts of cortisone.

The administration of cortisone was gradually decreased, and was discontinued in the course of one month. After discontinuation of cortisone (except for insulin) the patients were kept without any substitution therapy for about two to four weeks, during which time the completeness of the hypophysectomy was checked.

Our original intention was to keep the hypophysectomized patients on testosterone propionate, DCA, and thyroid hormone and to avoid cortisone because of its diabetogenic effect. Although some of the patients could get along rather well without cortisone, small doses of as little as 5 mg. per day (Table 3) considerably improved their well-being, especially the appetite, and thereby reduced the danger of hypoglycemia.

As has been mentioned, DCA caused vascular complications in 2 cases; it has to be given with caution in patients with renal damage. In the latter cases, reported in the addendum, we completely avoided DCA. This will probably make it necessary to give a maintenance dose of cortisone of 10–15 mg. per day.

Table 3 shows the daily insulin requirement before hypophysectomy and after the final adjustment.

Surgical procedure and postoperative complications

The *surgical procedure* was described in detail in 1953 (10) and has not been changed since. In the cases operated on later, there has been no evidence from the laboratory data of incomplete hypophysectomy; therefore, it has not been necessary to re-operate. In no case was intervention with respect to optic nerves or chiasm necessary at the operation.

It should be emphasized that the blood vessels of diabetic patients may not be normal, and that otherwise unimportant contusions of the frontal lobe due to operative trauma may in such patients lead to more hemorrhage and postoperative edema than would be the case in subjects with

TABLE 3. THE INSULIN REQUIREMENT BEFORE AND AFTER HYPOPHYSECTOMY IN 4 OF THE PATIENTS

Case No.	Daily requirement of insulin (units)		Maintenance substitution therapy after operation (in addition to 50 mg. of desicc. thyroid daily)
	before hypophysectomy	after hypophysectomy	
1	40 PZI+20 r.*	8 PZI	Implanted 300 mg. DCA† & 500 mg. T.P.‡
4	28 PZI+14 r.	6 PZI	Implanted 200 mg. DCA & 300 mg. T.P.
5	24 PZI	0	Implanted 200 mg. DCA & 300 mg. T.P. Cortisone, 5 mg. daily.
6	20+16 PZI	8 PZI	Implanted 200 mg. DCA & 300 mg. T.P. Cortisone, 5 mg. daily.

* PZI = protamine-zinc-insulin; r. = regular insulin.

† DCA = Desoxycorticosterone acetate pellets.

‡ T.P. = Testosterone propionate pellets.

normal blood vessels. The frontal lobe, which must be elevated in order to gain access to the pituitary fossa, must therefore be handled with the utmost care and even slight contusions from pressure of the brain spatula should be rigorously avoided. If the patient is hypertensive, which frequently happens, evacuation of fluid in the cisterna chiasmatis may not be sufficient to provide enough room. It may then sometimes be advisable to reduce the systolic blood pressure to 70–80 mm. of Hg by the injection of hexamethonium or similar drugs. Every effort should be made to prevent postoperative clots, and the dural and bone flaps should therefore be sufficiently devascularized to prevent the formation of an extradural clot. If the closure has been made in such a manner that an extradural clot cannot possibly occur, postoperative complications can be treated more conservatively than would be prudent after intracranial operations of other types. These patients do not react in quite the same manner as patients with brain tumors, and do not always regain consciousness promptly; or they may, after a short fairly lucid interval when recovering from the anesthetic, relapse again into a semicomatose condition. Re-elevation of the bone flap should be avoided if possible, and especially reopening of the dura. Due to the poor condition of the blood vessels, the brain of the diabetic patient is very vulnerable for the first few days following operation, and exploration for a suspected subdural clot may easily make a resection of the frontal lobe necessary.

Table 4 summarizes the postoperative complications in our cases. A serious complication has been *hypoglycemia*. Some hypoglycemic attacks have shown unusual features. They appeared three to four weeks after the operation, mostly following changes in the cortisone or insulin dosages. The attacks started with dizziness and slowly deepening coma, accompanied by cramps or epileptic fits. At this time the blood sugar level was

TABLE 4. MAIN POSTOPERATIVE COMPLICATIONS

Case No.	Severe acidosis	Severe hypoglycemia	Intracranial hematoma	Epileptic fits	Infection	Marked anemia	Anorexia, vomiting
1	−	−	−	−	−	+	−
2	−	−	−	−	−	−	−
3	−	+	−	+	origin unknown	−	−
4	−	+	(+)	+	−	+	+
5	−	−	−	−	−	+	+
6	−	+	−	+	pyelonephritis	−	−
7	−	−	−	+	−	+	−

lower than usual but always well above 100 mg. per 100 ml. The patients reacted promptly to a single administration of 20 ml. of 30 per cent glucose; but soon coma intervened again, without response to a similar dose of glucose. Only rapid intravenous infusion of glucose, continued for twenty-four hours or more, was able to save these patients. In spite of the large doses of glucose, the blood sugar level only slowly returned to normal values. The urine remained sugar-free for two to three days despite the administration of glucose.

In Case 3 the fatal outcome might be ascribed to a long-lasting hypoglycemia. The patient reacted to an initial single dose of hypertonic glucose, but not to a second dose. In this case we did not follow the foregoing procedure, and the patient died about twenty-four hours later in *status epilepticus*.

Four patients had epileptic fits during the postoperative course. In Case 4 they appeared in the second postoperative week, and disappeared after a small subdural hematoma was removed. In Cases 3, 4 and 6 the epileptic fits noted in Table 4 appeared in connection with hypoglycemic attacks. In Case 7 the intracranial vessels were very brittle, causing continuous bleeding during the operation. This necessitated removal of one frontal pole. This patient had several bouts of epileptic fits, which were influenced favorably by cortisone.

In Case 6 the postoperative course was complicated by pyelonephritis. This *infection* may have caused further impairment of renal function. In Case 3, the cause of the infection, which gave rise to high body temperature on discontinuation of the treatment with antibiotics, could not be found.

The *anemia* in these cases is probably due to removal of the hypophysis but also to the impairment of renal function. Transfusion of whole blood has been the main therapeutic measure.

In Cases 4 and 5, *anorexia* was an obstacle in the management. Anorexia may be relieved to a great extent by 5–10 mg. of cortisone per day.

Tests for the completeness of the hypophysectomy

The authors believe that the hypophysis must be removed completely in order to obtain a therapeutic result. It was, therefore, important to know as soon as possible after the operation, whether the hypophysectomy was complete. Until now, the available methods for confirming total removal have been dependent upon demonstration of adrenocortical and thyroid insufficiency. It is conjectural whether the lack of an eosinopenic response to adrenaline is proof of the completeness of the hypophysectomy. In the present series it was often necessary to administer cortisone for long periods of time, thus making it difficult to evaluate adrenocortical function.

Table 5 shows results of the tests performed in an attempt to elucidate this question. *The adrenaline test* was used several times. In 2 cases (Nos. 5 and 6) there was still an eosinopenic response two weeks after the hypophysectomy. In all other instances the results of the adrenaline test were negative. This is in agreement with our experience in hypophysectomized patients with malignant diseases. *The excretion of 17-ketosteroids* fell to low or zero values within three to six weeks after the operation. In 2 cases *the excretion of corticoids* was measured postoperatively and found to be zero. The *BMR values* became subnormal in all cases. In Case 6, however,

TABLE 5. TESTS OF FUNCTION OF THE ADRENAL CORTEX AND THE THYROID AFTER HYPOPHYSECTOMY

Case No.	Time after hypophysectomy	Adrenal cortex						Thyroid gland					
		Adrenaline test (eosin./cu. mm.)		ACTH test (eosin./cu. mm.)		Urinary 17-KS (mg./day)	Urinary corticoids (mg./day)	BMR %	Serum cholesterol (mg./100 ml.)	Test with I^{131}			
		before	after 4 hrs.	before	after 4 hrs.					Thyroidal uptake after 24 and 48 hrs. (%)	Urin. excret. after 24 and 48 hrs. (%)	Thyroidal clearance (ml./min.)	Renal clearance (ml./min.)
1	2 wks.					4.0–5.0							
	3 wks.					2.5							
	4 wks.			756	485				312–336				
	5 wks.					0.7–1.4		−12					
	7 wks.	377	330	340	231			−15					
	8 wks.		333					−15					
	10 wks.	357		316	393			−20					
	14 wks.	1370	1050	1400	1325				338				
	15 wks.	975	813										
3	0	760	360	500	100	7.7–4.2		−3	324–274				
	1 wk.	943	860			1.0			278				
	3 wks.					0.6			216				
	4 wks.							−30					
4	3 wks.	465	520			0							
	4 wks.	588	477			0							
	6 wks.							−19					
	9 wks.									0–0	11–11	0	4
5	2 wks.	119	66			2.2–3.5							
	3 wks.					0.3–1.8							
	5 wks.					0.7			372				
	6 wks.					0		−43					
6	2 wks.	312	180										
	3 wks.					3.6–5.4		−15	377				
	4 wks.	231	227										
	5 wks.					1.1–3.4							
	8 wks.	305	200			1.0		−6					
	10 wks.					0				14–	36–49		
	9 mos.	311	320				0	−10	390–508	0–0	33–15	0	12
7	3 mos.	670				0							

the decrease was insignificant, whereas the test with *radioiodine* showed a thyroidal clearance of zero.

End-results in living patients

General condition. The patient in Case 1 is living the ordinary life of a blind man two and a half years after hypophysectomy. He is up and about all day. According to his own opinion there has been no further deterioration of his general well-being. He has tolerated common colds well. He eats all foods except pure sugar. He has remarried and maintains a normal sexual life. His appetite is very good, and he maintains his normal body weight.

The patient in Case 5 was hospitalized for one year after the hypophysectomy, mainly because of nephrotic edema. She left the hospital in May 1954, has had no edema, and is up and about most of the day. She has had periods of anorexia and weight loss, but at present maintains her body weight.

The patient in Case 6 has preserved her preoperative vision. She is helping in the household work and is up and about all day. She has a good appetite, eats all kinds of food, and maintains her body weight without edema.

Renal function. The renal clearances before and after the operation are shown in Table 6. In Cases 1, 4, 5 and 7 the renal plasma flow remained unchanged after the hypophysectomy. The same held true for the effective renal blood flow in Cases 1, 5 and 7, whereas in Case 4 the renal blood flow had decreased at the last examination. However, at this examination the patient had a marked anemia. In Case 6 the renal plasma flow and renal blood flow decreased significantly after the operation, probably because of a subacute pyelonephritis. The glomerular filtration rate was unchanged in Case 1 during the two and a half years after hypophysectomy. In Cases 4, 5 and 6 there was some decrease during the postoperative period.

As for the albuminuria, it disappeared after hypophysectomy in Cases 1 and 4 but remained in the other cases.

The level of serum nonprotein nitrogen remained normal during most of the postoperative period. During periods of vomiting and dehydration, however, the NPN values rose to 50–70 mg. per 100 ml. in Cases 4 and 5. At the time of discharge from the hospital all patients had normal NPN values.

Vision. The patients in Cases 1, 4, 5 and 7 were amaurotic or almost amaurotic before hypophysectomy, and no change in either direction was observed. The cataracts and eye-ground changes remained unchanged. In Case 6 no further progress of the retinopathy has been noted during the first postoperative year.

TABLE 6. CLEARANCE STUDIES BEFORE AND AFTER HYPOPHYSECTOMY IN 5 CASES OF DIABETES MELLITUS

Case No.	Date of test	Date of hypophysectomy	GFR (ml./min.)	RPF (ml./min.)	FF	RBF (ml./min.)	BP (mm. Hg)	Postop. hormone therapy
1	July 1950		152*				140–165/70–105	
	Jan. 1951		76*				150–180/90–110	
	Nov. 1951		75*				175–190/80–110	
		Nov. 1951						
	July 1952		55	342	0.16	526	130/80	DCA+TP+thyroid
	July 1952		49	362	9.14	540	135/80	DCA+TP+thyroid
	Dec. 1952		69	438	0.16	730	140/100	DCA+TP+thyroid
	Apr. 1953		63	353	0.18	504	130/90	DCA+TP+thyroid
	Jan. 1954		71	339	0.21	506	130/90	DCA+TP+thyroid
4	Jan. 1953		60*	216			150/105	
	Mar. 1953		69*					
		Mar. 1953						
	Apr. 1953		30	232	0.13	387	140/80	DCA 5 mg.
	June 1953		36	197	0.18	308	125/90	DCA+TP+thyroid
	Feb. 1954		22	201	0.11	264	140/90	DCA+TP+thyroid
5	Nov. 1950		42	285	0.15		120/80	
	Mar. 1953		(42)	261	0.16	458	145/90	
		Apr. 1953						
	Sept. 1953		62	272	0.23	394		Cortisone 5+thyroid
	Oct. 1953			373			130/90	DCA+TP+cortisone 5+thyroid
	Feb. 1954		35	363	0.10	558	130/90	DCA+TP+cortisone 5+thyroid
	Feb. 1954		32	322	0.10	460	130/90	DCA+TP+cortisone 5+thyroid
6	May 1953		76	339	0.22	565	145/100	
		May 1953						
	Oct. 1953		43	188	0.23	313	145/100	DCA+TP+thyroid
	Jan. 1954		41	258	0.16	391	115/85	DCA+TP
7	Nov. 1953		68	453	0.15	676	220/110	
		Dec. 1953						
	Mar. 1954		55	434	0.13	689	160/90	Cortisone 5+DCA+TP+thyroid

GFR = glomerular filtration rate; RPF = renal plasma flow; FF = filtration fraction; RBF = renal blood flow.
* Creatinine clearance.

Blood pressure. In all instances the blood pressure became normal after hypophysectomy. Hypotensive levels were reached in Cases 4 and 5 during the early postoperative period; the blood pressure was corrected by administration of DCA. As mentioned previously, the implantation of DCA in Case 4 was followed by an acute elevation of blood pressure.

DISCUSSION AND CONCLUSIONS

The present data show that hypophysectomy can be performed in cases of severe diabetes mellitus with progressing vascular complications, and that this operation can favorably influence the diabetic state. As expected, hypophysectomy in our series increased insulin sensitivity, thus permitting a decrease in insulin dosage.

The primary aim in performing hypophysectomy was to stop the progress of the vascular complications. It is at present difficult to appraise the

results in this respect, as this first group of hypophysectomized diabetics consisted mainly of patients with far advanced renal and retinal changes. However, in Case 6—the only surviving patient in whom the rapidly progressing retinopathy had not advanced to amaurosis before operation—the retinal changes have not shown any progress during the first postoperative year.

A longer period of time is necessary for observation of the effect of hypophysectomy on renal function. Appraisal has therefore been possible in only 1 patient (Case 1), in whom the clearance values are unchanged two and a half years after the operation. In this connection it is worth mentioning that in subjects with intact renal function, hypophysectomy may induce a significant decrease in the glomerular filtration rate and renal plasma flow (data to be published). This fact influences the interpretation of the postoperative clearance values in diabetics. The clearance of PAH in these cases must also be interpreted cautiously, as the renal extraction of PAH was not determined.

The elevated blood pressure in our patients decreased to normal levels following hypophysectomy. This may be looked upon as a favorable sign. In 2 cases a long-standing albuminuria also disappeared.

SUMMARY

Hypophysectomy was performed in 7 cases of severe diabetes mellitus with progressive malignant vascular complications. Three of these patients are alive from one to two and a half years after the operation. In addition, hypophysectomy was performed in 2 diabetics who have been followed postoperatively for two months.

Following removal of the hypophysis, insulin sensitivity increased. Thus, insulin dosage could be markedly decreased.

The effect of the operation on the vascular complications is difficult to appraise, since the retinopathy in all but 1 case was far advanced and irreversible. In 1 case there was no progression of retinopathy in a period of one year following hypophysectomy. In the patients who were hypertensive, the blood pressure became normal after the operation, and in 2 patients albuminuria of long standing disappeared.

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ALTERATIONS IN WATER AND SALT METABOLISM AFTER BILATERAL ADRENALECTOMY IN MAN*†

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INVESTIGATIONS into the mechanisms of adrenal insufficiency have focussed on salt and water metabolism since Bauman and Kurland (1) first demonstrated electrolyte changes in the serum of adrenalectomized cats. The urinary loss of salt and the therapeutic effects of a high-salt diet or of desoxycorticosterone acetate in adrenal insufficiency in man and animal (2–7) led to the concept that one function of the adrenal cortex is to protect against the renal depletion of body sodium. Additional studies in animals emphasized this point of view by indicating a reduction during adrenal insufficiency of plasma volume (8) and extracellular fluids (9–15). Analysis of tissues secured during adrenal insufficiency indicated changes in the intracellular constituents as well (12, 15–19). The concept has been revised to include as part of the syndrome the movement of water and potassium intracellularly which, in combination with renal loss of sodium and retention of potassium, leads to further reduction in the extracellular fluids (20). This, in turn, results in circulatory embarrassment and decreased renal function, and is rapidly fatal.

In 1931, Rowntree and Snell (2) first called attention to the lack of water diuresis in the Addisonian patient given a water load. This was followed by the development of the water test for adrenal insufficiency (22), and the demonstration of antidiuretic substances in the body fluids of animals with adrenal insufficiency (23, 24). Slessor demonstrated the persistence of an antidiuretic substance in human serum and urine in adrenal insufficiency in

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spite of solute dilution by water (25). He found that after administration of cortisone, a water load was followed by an effective water diuresis and disappearance of the antidiuretic substance.

The demonstration by Pearson (26) that water retention could play a prominent part in the response of the adrenalectomized human to cortisone withdrawal was consistent with the concept of a loss of diuretic capacity, but was difficult to resolve with that of depletion of body fluids and electrolytes. The present study was therefore set up to examine the effects of cortisone withdrawal in the adrenalectomized patient by using metabolic balance methods combined with the determination of volumes of distribution of inulin, antipyrine, and radioactive sodium. The results obtained are at variance with the experimental data in animals, and the classic concept of the salt-losing Addisonian patient.

PROCEDURE

Two patients were tudied in detail.

GMc, a 39-year-old woman, had metastatic carcinoma of the breast, predominantly to bone. The metastases were first demonstrated two years following her second radical mastectomy. Significant, but transient remissions had been induced by testosterone propionate and later by oophorectomy. One month prior to this study she underwent complete bilateral adrenalectomy. Following an uneventful postoperative course she had a striking symptomatic remission. Maintained on 75 milligrams of cortisone daily by mouth as the only substitution therapy, she was fully ambulatory and without symptoms. By physical and roentgen examination she was free of abnormal fluid deposits. She had normal tolerance to water loading, fasting, glucose and insulin.

MC, a 69-year-old man, had a five-year history of adenocarcinoma of the prostate, with skeletal metastases. He had been treated by transurethral resection and erratically administered estrogens. Two months prior to the present study he had undergone bilateral adrenalectomy, with no subsequent improvement in bone pain. One month later, he responded well to orchiectomy, and on 50 milligrams of cortisone daily by mouth was ambulatory and comfortable. The results of water, insulin, and fasting tolerance tests were normal. A glucose tolerance curve was somewhat of the diabetic type. Two weeks prior to, and throughout this study he received daily 5 milligrams of testosterone propionate intramuscularly In spite of a rising level of serum acid phosphatase, there was no return of his bone pain. He showed no evidence of abnormal fluid accumulation.

The patients were on a metabolic ward, receiving an analyzed, constant low-sodium diet with a daily oral supplement of sodium chloride. After a six-day control period, cortisone was withdrawn for as long as the patient's condition allowed (three to four days). A final control period on the same cortisone dosage as the first then followed. During each control period, and on the last day of the withdrawal, the volumes of distribution of inulin, radioactive sodium, and antipyrine were determined. In Patient GMc, analyses were made of 24-hour urine and six-day stool collections for sodium, potassium and nitrogen. In Patient MC, 24-hour urine measurements were made for sodium and potassium. Daily body weights under standard conditions were determined, as well as frequent hematologic and blood chemical values (Tables 2 and 3). Cortisone was given orally in 25-milligram doses. Drinking water was unrestricted. When food

intake could not be maintained during the withdrawal of cortisone, the daily sodium chloride ration was given intravenously. Uneaten food was analyzed daily in order to determine the intake accurately.

Four other adrenalectomized patients were studied during five cortisone withdrawal periods while on a metabolic balance regimen.

METHODS

The dilution techniques were standardized for each patient with regard to equilibration time, dosage, sampling and calculations. The antipyrine method of Soberman *et al.* (27) was used. Four to five blood samples were taken, the first being at least three hours after injection. Radioactive sodium (Na^{24}) was prepared for injection and given intravenously in doses of 200 to 400 microcuries. A 14-hour period of equilibration was used in Patient GMc, and a 24-hour period in Patient MC. Urinary excretion of Na^{24} was measured and a correction applied. Gamma-ray counting to 2 per cent accuracy was carried out in duplicate with a Texaco tube, and the same samples were analyzed chemically for sodium with a flame photometer. Total exchangeable sodium was calculated as described by Forbes and Perley (28). The technique of Gaudino, Schwartz and Levitt (29) for inulin distribution was followed, with the Roe, Epstein and Goldstein method of inulin analysis (30). The inulin infusion was run for twelve hours in Patient GMc and for eight hours in Patient MC. Plasma inulin levels of at least 30 milligrams per 100 ml. were obtained. Although attempts were made to determine the inulin space by calculating the difference between the quantities infused and the urinary excretion up to the cessation of the infusion, these figures are not presented, since they invariably yielded excessively large and erratic volumes of distribution. This was an arithmetical magnification of the fact that total recoveries of inulin ranged from 90 to 98 per cent, and is in part an expression of the relatively long periods of infusion. Inulin clearance was derived from the dilution data by the constant infusion principle used by Berger *et al.* (31), and was corrected to 1.73 square meters of surface area.

The remaining chemical methods are described in a previous publication from this laboratory (32).

RESULTS

In Figure 1 the serum sodium level, sodium balance, potassium balance, body weight, and fluid intake and output are charted for Patient GMc. The volumes of dilution for inulin and antipyrine, and the total exchangeable sodium are given in Table 1, and in Table 2 the pertinent hematologic and blood chemical values are listed.

TABLE 1. VOLUMES OF DILUTION FOR PATIENTS GMc AND MC

	GMc			MC		
Experimental days	2	9	14	1	9	17
Inulin space (liters)	9.8	11.9	8.7	7.5	8.6	6.6
Antipyrine space (liters)	25.8	26.2	25.4	24.3	23.8	23.3
Total exchangeable sodium (mEq.)	1,471	1,560	1,569	1,648	1,580	1,775

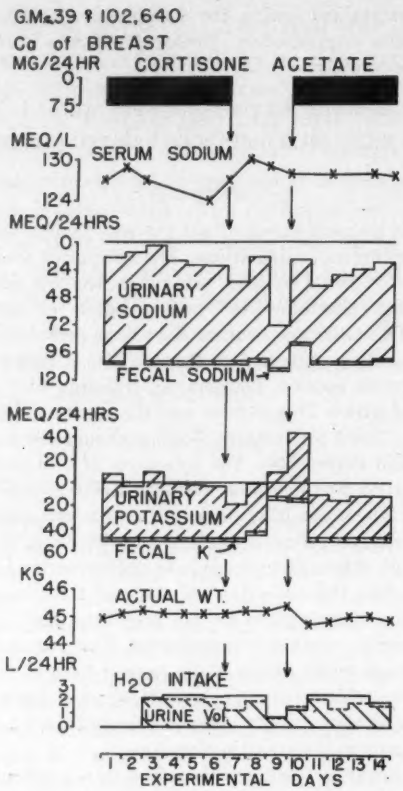

FIG. 1. *Effect of cortisone withdrawal on adrenalectomized Patient GMc.* Sodium and potassium intake are plotted from the zero line down, and urinary and fecal excretion from the intake line up. The broken line represents 24-hour urine volume.

On the second day after withdrawal of cortisone, the patient became weak and anorexic, and she appeared washed out. On the third and last day without hormone she complained of severe malaise, weakness, muscle aches and pains and profound anorexia and nausea. She was bed-ridden and looked gaunt and pallid. The blood pressure fell to 86/58 mm. of Hg, and the patient appeared critically ill. Within twenty-four hours of resumption of cortisone there was dramatic clinical improvement with a return of appetite, strength and ability to walk.

Sodium balance, which was slightly positive in the control period, became more positive during cortisone withdrawal, and remained slightly positive during the recovery period. The serum sodium level was below normal initially and did not change significantly throughout the study. The total exchangeable sodium measured after three days of cortisone with-

TABLE 2. BLOOD CHEMICAL AND HEMATOLOGIC DATA IN PATIENT GMc

Day	Tot. prot. (Gm. %)	Urea nitrogen (mg./ 100 ml.)	NaCl (mEq./L)	K (mEq./L)	CO_2 (mEq./L)	Fasting sugar (mg./ 100 ml.)	Hgb. (Gm. %)	Hematocrit (%)	WBC (1,000 per cu. mm.)	Lymph. %	Eos. per cu. mm.
1			97	5.4	26.1		12.7		8.6	36	108
2	7.2	18.3	103	5.1	25.7	82	12.7	38	9.4		181
3			92	5.1	26.2		12.1		10.1	26	103
6	6.4	15.1	95	5.6		48	11.9	33	10.7	35	206
7	6.8	14.8	100	5.7	25.8	68	11.3	33	10.2		328
Cortisone administration ended on day 7											
8	6.4	17.7	98	5.6		70	9.5	33	9.4		281
9	6.0	10.1	95	5.2	26.1	74	10.4	33	8.0	44	209
10	6.4	12.3	95	6.0	24.7	77					
Cortisone administration begun on day 10											
11									8.1		26
12				5.8	25.1						
13	6.8	9.9	101			80	9.1	30	9.0	31	103
14	6.4	12.4	103	6.0	25.1	77	9.4		10.4		91
15	6.0	9.6	102	6.0	25.0	87	8.9	27	13.0		69
16	6.9	13.7	98	6.3	25.3	87	9.8	29	13.7	22	75

drawal increased 89 mEq. over the control value and remained the same on the fifth day of recovery.

Body weight increased 0.3 Kg. during the three-day period of cortisone withdrawal, in spite of a marked reduction in food and water intake. On the third day of withdrawal the urine volume declined and inulin clearance decreased from 122 to 102 ml. per minute. The antipyrine space was unchanged, within the limits of the method. The inulin space increased by 21 per cent— from 9.8 to 11.9 liters.

During the first twenty-four hours of resumption of cortisone, urine output exceeded water intake, and a loss of 0.7 Kg. in body weight occurred. On the fifth day of recovery, the inulin clearance had returned to the previous control value of 122 ml. per minute. The antipyrine and inulin spaces had declined to below the initial control levels.

Urinary potassium declined significantly on the first day of cortisone withdrawal and rose sharply on the first day of resumption of cortisone. The serum potassium level increased slightly during cortisone withdrawal and remained elevated during the recovery period. Nitrogen balance followed the same daily trends as did the potassium balance. From a positive balance of 1.2 grams per day during the control period, it fell progressively to 2.7 grams of negative balance on the first day cortisone was resumed, and then returned to a positive figure.

The percentage of lymphocytes rose during cortisone withdrawal and fell to control values during recovery. The eosinophil count fluctuated

erratically. Hemoglobin and hematocrit values gradually fell, probably as a cumulative result of analytic blood lettings.

Figure 2 illustrates the data on Patient MC for serum sodium concentration, sodium balance, water intake, urine output, and body weight. The volumes of dilution are given in Table 1. In Table 3 the pertinent hematologic and blood chemical values are recorded.

On the second day of withdrawal of cortisone, the patient began to complain of dizziness on standing. On the following day he became progressively more depressed, irritable, weak and anorexic. He was unable to leave his bed and, in the final hours of withdrawal, nausea and retching developed. Twenty-four hours after resumption of cortisone, he was subjectively improved, and in subsequent days he rapidly returned to his previous clinical state.

The serum sodium level, which was normal during the control period, fell to 119 mEq. per liter on the third day of cortisone withdrawal but re-

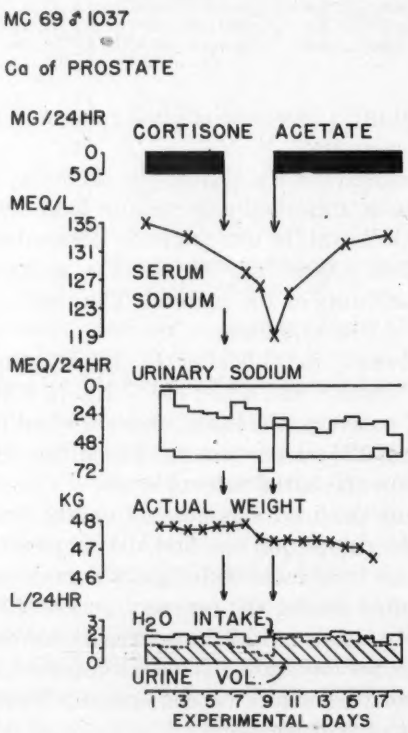

FIG. 2. *Effect of cortisone withdrawal on adrenalectomized Patient MC.* Sodium intake has been plotted from the zero line down, and urinary excretion from the intake line up.

TABLE 3. BLOOD CHEMICAL AND HEMATOLOGIC DATA ON PATIENT MC

Day	Tot. prot. (Gm. %)	Urea nitrogen (mg./ 100 ml.)	NaCl (mEq./L)	K (mEq./L)	CO_2 (mEq./L)	Fasting sugar (mg./ 100 ml.)	Hgb. (Gm. %)	Hema-tocrit (%)	WBC (1,000 per cu. mm.)	Lymph. (%)	Eos. per cu. mm.
1	6.6	32.0	106	5.6	18.8	79	10.6		9.4	22	125
3							10.4	33			74
4	6.4	31.8	108		19.6	91					
5							10.2	34	13.6	22	25
Cortisone administration ended on day 6											
8		41.0	101	5.8	19.2	86	10.9	33	10.3	39	44
9				6.0			11.5	34			63
10	6.6	33.8	98	5.9	19.2	109	11.3	35	16.5		63
Cortisone administration begun on day 10											
11	6.4	29.0	94	5.6	22.3	153	10.0	32	8.2	32	69
12							10.9	32	10.5	23	31
15	7.4	47.6	91	6.0	19.2	99	10.0	31	12.3	25	50
18	6.8	40.0	102	5.4	16.5	86	10.0	30	10.0	23	19

turned to nearly normal on the fifth day after resumption of cortisone. The sodium balance remained positive throughout. The total exchangeable sodium decreased 68 mEq. after three days of cortisone withdrawal and rose to 127 mEq. over the control value on the seventh day of resumption of cortisone.

During the three-day period of cortisone withdrawal, body weight decreased 0.4 Kg. Urine volume declined steadily. The inulin clearance, which was low initially (61 ml. per minute) fell to 52 ml. per minute. There was an insignificant decrease in antipyrine space. The inulin space expanded 15 per cent, from 7.5 to 8.6 liters.

During the first twenty-four hours of cortisone resumption, the urine volume rose, but body weight did not change. On the eight day of recovery, the inulin clearance remained low (51 ml. per minute). The antipyrine and inulin spaces had declined to below the initial control levels.

The concentration of serum potassium did not change significantly throughout. The blood urea nitrogen level was elevated and the serum CO_2 content was below normal throughout the period of observation. The serum chloride levels paralleled those of the serum sodium.

The percentage of lymphocytes rose during cortisone withdrawal and fell to control values during recovery. The eosinophil counts fluctuated erratically. There was a slight rise in hemoglobin and hematocrit levels during cortisone withdrawal, and a decline during recovery.

In Table 4 the pertinent data have been summarized in 5 cortisone withdrawal experiments on 4 additional patients adrenalectomized for advanced cancer. Cortisone was the only hormone replacement used. Severe

clinical deterioration was the determining factor in each case for resuming the cortisone therapy.

DISCUSSION

Vulnerabilities inherent in the methods make the results of this study subject to qualifications. Insensible losses of sodium and water were not included in the balance measurements. Adrenal hormones are known to affect sweat composition (33). It is possible that the discrepancy between sodium balance and total exchangeable sodium during withdrawal of cortisone in Patient MC was related to non-urinary losses of sodium. The lack of fever and noticeable sweating, and the fact that he passed no stool during the entire withdrawal period minimize this possibility. An alternate explanation for the discrepancy may involve a change in the fraction of total body sodium available for exchange (34). There was also no fever or sweating during the study in Patient GMc, and here the balance and dilution results for sodium were comparable with each other.

The technique used for inulin distribution does not correct for the inulin present in the renal dead space. Provided the urine volume is high, or at least constant, this defect may not be significant. However, during the cortisone withdrawal measurements, oliguria was a constant occurrence, and

TABLE 4. SUMMARY OF 5 CORTISONE WITHDRAWAL EXPERIMENTS

Patient	I.B.	J.WM.	J.WM.	M.D.	J.WE.
Age	65	66	66	54	35
Sex	M	M	M	F	M
Duration of withdrawal (days)	3	3	3	4	7
Control serum Na (mEq./L)	138	138	131	128	125
Withdrawal serum Na (mEq./L)	124	137	131	120	118
Change in Na balance during withdrawal (mEq./day)	0	−60	−150	0	−25
Control serum K (mEq./L)	4.6	3.0	4.8	5.0	5.5
Withdrawal serum K (mEq./L)	4.1	3.2	4.9	5.5	5.5
Change in K balance during withdrawal (mEq./day)	−40	0	0	0	
Net K loss after cortisone started (mEq.)		10	30	0	
Wt. change during withdrawal (Kg.)	+2.4	0	−1.5	+0.4	−0.5
Wt. change during first 3 days after restarting cortisone (Kg.)	−3.3	−2.7	−1.2	−1.6	−1.0
Change in serum CO_2 during withdrawal (mEq./L)		0	0	−5	−5
Change in serum chloride during withdrawal (mEq./L)		−1	−3	−10	−10
Change in blood urea N during withdrawal (mg./100 ml.).		0	−7	+2	

was greater in extent than the reduction in inulin clearance. In Patient GMc a false increment to the inulin space of 150 ml. for each ml. of renal dead space was approximated during withdrawal, whereas this value was roughly halved during control measurements. In the human, the relationship between the volume of the renal dead space and the rate of urine flow is too poorly understood to warrant a calculated dead-space correction. In the study of Patient MC an effort was made to keep the urine flow constant by producing a minimal osmotic diuresis at the end of each inulin-space determination by the intravenous infusion of 12.5 Gm. of mannitol in 200 ml. of water over a thirty-minute period. A urine flow of about 2 ml. per minute was consistently achieved. Larger amounts of osmotically active infusions can cause gross shifts in internal fluid distribution (35, 36), and adrenal insufficiency is known to decrease the compensatory adjustments to water loading (37, 38, 39). It is realized that this is an uncertain substitute for oliguria, but an alternative was not apparent.

Consideration of the inulin space, or any similar volume of distribution, introduces a problem of interpretation. The validity of the equation of inulin space to extracellular fluid volume, if controversial in the normal subject (40, 41), becomes totally enigmatic in adrenal insufficiency. An increase in inulin space in adrenal insufficiency may represent expansion of the extracellular fluid volume (or an analogous readily diffusible volume of similar size) or, conversely, may indicate alterations in permeability allowing inulin access across cell membranes or into more remote connective-tissue interstices from which it was previously excluded (41, 42). The adrenal hormones are known to be involved in gross tissue permeability (43, 44). It is interesting to speculate whether the permeability changes that are responsible for the increase in thiocyanate space during experimental pyrexia (45) are a function of the adrenal cortex. If so, then the thiocyanate studies in adrenal insufficiency showing a reduced volume of distribution (9, 10, 12–15) may actually be measuring a reduction in permeability (opposite to the effect of pyrexia).

Steroid metabolism in the cancer patient is known to differ from the normal (46). The possibility exists that the response to adrenalectomy and to cortisone in the patients in this study, all of whom had cancer, was unique and cannot be equated to comparable circumstances in the noncancerous subject. It is also conceivable that the absence of gonadal secretions, and in 1 case the administration of testosterone propionate, were modifying factors.

In Patient GMc the development of clinical adrenal insufficiency after cortisone withdrawal was associated with a minimum of measurable change. The serum sodium level did not fall. There was no indication of sodium loss, or of hemoconcentration, in spite of a fall in blood pressure

and a 30 per cent reduction in glomerular filtration rate. The conventional changes in potassium balance were seen only on the first day of withdrawal and on the first day of resumption of hormone, and then were minimal. There was a suggestion of some water retention during insufficiency, but no more than a fraction of a liter. The inulin space was the only measurement that showed a pronounced change, and it is made suspect by the potential dead-space error.

In Patient MC adrenal insufficiency was associated with definite alterations in salt and water metabolism. The progressive fall in serum sodium concentration following cortisone withdrawal, and the subsequent reestablishment of control levels after hormone replacement were striking. That this was not due to urinary sodium losses is clear from the balance data. The measured expansion in inulin space was numerically equivalent to the fall in level of serum sodium, whereas the decrease in exchangeable sodium was not, of itself, great enough to account for the change in serum concentration. Since there was no indication of water retention in Patient MC, the data (subject to the reservations already discussed) indicate that during cortisone withdrawal there was a redistribution of body fluids such that the extracellular space expanded at the expense of intracellular volume, resulting in a secondary dilution of extracellular sodium concentration. In spite of this apparent expansion, there was postural hypotension a suggestion of hemoconcentration, and a fall in the glomerular filtration rate.

The syndrome of renal salt-wasting, hyponatremia, dehydration, hemoconcentration, and hyperkaliemia did not occur in its entirety in any of the 7 cortisone withdrawal experiments described. In terms of salt and water metabolism, there was no one consistent pattern that could be associated with the inevitable clinical collapse that follows omission of cortisone in the adrenalectomized patient. The response in 1 patient (I. B.) was characteristic of water retention and intoxication, whereas this phenomenon in most of the patients was a suggestion only. Evidence for hemoconcentration was found twice; hemodilution was suggested twice (not including I. B.). The data indicated that any retention secondary to diminished renal function during withdrawal of cortisone was balanced by the reduction in food intake. In the 3 instances in which it occurred, sodium diuresis was not associated with an equivalent loss of weight. There was no evidence of renal salt-wastage in 4 patients, and no fall in the serum sodium level in 3.

The mechanisms, by means of which the absence of adrenocortical hormones becomes so crippling, are not to be found in these results. However, there are apparent in these data several refutations of mechanisms currently considered valid. It has been demonstrated that (a) clinical insufficiency

can occur without salt wastage or a reduction in the concentration of serum sodium, (b) the hyponatremia of adrenal insufficiency may be secondary to, or associated with, the expansion of extracellular fluids, and (c) during insufficiency the reduction of glomerular filtration rate and of blood pressure can occur without being secondary to hemoconcentration or loss of extracellular fluids and body water.

The disparity between the results of these studies and the studies already quoted on experimental animals, or the reports on Addisonian patients (20, 47–49), may hinge upon the nature of the adrenal replacement used. Provided salt is not restricted, cortisone in the dosages used here constitutes clinically and physiologically adequate replacement therapy for the adrenalectomized patient (50, 51). For this reason, a salt-retaining component was not added to the control medications in the present study. It is possible that such a regimen leaves the patient deficient in a sodium-retaining factor (52). The low control level of serum sodium and the high level of serum potassium in Patient GMc may be a reflection of this. However, her total exchangeable sodium in the final control period was only 2 mEq. per Kg. less than that determined prior to adrenalectomy, and it was within two standard deviations of the mean for females defined by Forbes and Perley (28). In addition she has continued to maintain such levels of serum sodium for nine months since this study was performed, without deleterious effect. The final exchangeable sodium value for Patient MC was within one standard deviation of the mean for males, as expressed per Kg. of body weight (28). On the other hand, by using an agent such as desoxycorticosterone acetate, for which the line between physiologic and pharmacologic action is uncertain, the investigator may be unwittingly setting the stage for an artificial withdrawal response. In contrast to the procedure employed in this report, all the studies referred to in the literature involve either the acutely postoperative subject or subjects undergoing replacement therapy, which included desoxycorticosterone acetate, adrenocortical extract, or large doses of salt. The final outcome of this controversy in all likelihood will be a subtle compromise; but until the outcome is known, all adrenalectomy experiments depending for control upon a supposedly normal adrenal replacement, are subject to reservation.

The observations of Hills *et al.* (53) in a series of hormone-withdrawal studies in patients following subtotal adrenalectomy for advanced hypertension, deserve special mention. Balance measurements were made and volumes of dilution determined in 7 patients before and during withdrawal of hormone. Four patients received a combination of cortisone and desoxycorticosterone acetate, and 1 received cortisone with a sodium intake equivalent to over 14 Gm. of salt per day. The 2 remaining patients received

cortisone alone and were maintained on oral doses of only 8 and 9 mg. daily, respectively. Presumably, therefore, they had significant amounts of residual adrenal function. The inulin space results were subjected to a correction based on a hypothetical 30-ml. volume for the renal dead space. Since they found no change in inulin clearance during adrenal insufficiency, such a correction becomes very sensitive to potential volume changes in the dead space secondary to changes in the rate of urine flow. Urine flow values were not given with their data. However, a pronounced decrease in flow has been a constant finding in the cortisone withdrawal experiments of the present report. If a decrease in the dead-space volume actually occurred between the control and withdrawal inulin-space measurements, the correction used would produce an artefactual decrease in the withdrawal value. The pooled results of the hypertensive series yield a decrease in inulin space after hormone withdrawal of 2.6 liters (26 per cent). The results are biased, however, by the group receiving desoxycorticosterone acetate, since each showed a decrease greater than the mean. In view of the difference in experimental subjects (hypertension *vs.* cancer; subtotal *vs.* total adrenalectomy), in the type of adrenal replacement therapy, and in the technical details of the measurements, these two studies are neither directly comparable nor contradictory.

It is pertinent to mention the significance of our results in terms of the management of acute adrenal insufficiency in the adrenalectomized patient. An agent such as intravenous cortisone, which provides rapid adrenal hormone replacement, is the crux of treatment in these cases. The management of water and electrolyte therapy must be highly individualized. Vigorous administration of saline should be weighed against the risk of pulmonary edema, since the patient may already have an expanded extracellular volume or an excess of body water. In the treatment of hypotension, vasoconstrictants have the priority, unless there are specific indications of an absolute deficiency of circulating blood volume.

SUMMARY

1. Six bilaterally adrenalectomized cancer patients have been studied during cortisone withdrawal, to determine the alterations in salt and water metabolism in acute adrenal insufficiency. Metabolic balance measurements of water and electrolytes were carried out in all patients, and in 2 subjects the volumes of distribution of inulin, antipyrine and radioactive sodium were also determined. Profound clinical collapse occurred in all patients within three to seven days after withdrawal of cortisone.

2. In 7 withdrawal studies, water retention was the most frequently observed metabolic change.

3. In the 2 patients studied, there was an apparent expansion of the inulin space of 15 per cent and 21 per cent, respectively, during cortisone

withdrawal, without significant alterations in antipyrine space or in total exchangeable sodium.

4. Hyponatremia developed in 4 subjects, unassociated with significant renal salt-wastage.

5. Serum potassium levels rose slightly in 2 patients and remained unchanged in 4 patients, during cortisone withdrawal.

6. Although internal and external changes in salt and water metabolism have been observed, these results tend to minimize the role of these phenomena in the pathologic physiology of acute adrenal insufficiency in man.

7. These data are considered unique, in that a specific salt-retaining factor was not included in the control medications.

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SOURCES OF ERROR IN CLINICAL BIOASSAYS OF SERUM CHORIONIC GONADOTROPIN

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ESTIMATES of the serum concentration of human chorionic gonadotropin (H.C.G.) in international units throughout normal and abnormal pregnancy have been made by several workers using different bioassay methods (1–8). Implicit in these estimations are the assumptions 1) that the hormone present in the serum of pregnant women and the international standard preparation are qualitatively identical, and 2) that other constituents of serum do not contribute in any way to the effect of H.C.G. on the target organs. However, it has been recently reported that the effect of H.C.G. on the rat uterus and ovary is greatly increased when the hormone is administered in human plasma instead of in saline (9, 10, 11). Since this observation appeared to cast doubts on the validity of estimates of serum H.C.G. by these two methods, it was felt necessary to ascertain whether results obtained by the prostatic weight and seminal-vesicle weight methods could be criticized on the same grounds.

In this paper, therefore, a comparison is made in two different laboratories of the biologic activity of H.C.G. dissolved in serum or plasma with that of H.C.G. dissolved in saline, using a variety of bioassay techniques.

MATERIALS AND METHODS

A. *Assay material*

Samples of serum or of heparinized plasma were obtained from normal men, from men suffering from nonendocrine diseases, and from normal women during reproductive life. In one assay, rabbit serum was used.

The International Standard for H.C.G. was employed in all experiments. In the assays subsequently reported, H.C.G. dissolved in saline is referred to as the "standard" preparation (S) and H.C.G. dissolved in serum or plasma as the "unknown" preparation (U). The total volume of fluid (saline, plasma or serum) administered to each rat over the three or four day injection period is indicated in each assay.

B. *Methods of assay*

Assays of H.C.G. were performed (a) in intact immature male rats, (b) in hypophysectomized immature male rats, and (c) in intact immature female rats.

(a) In *intact immature male rats* the following criteria were used:

1. Increase in prostatic weight (12).
2. Increase in seminal-vesicle weight (12, 13).
3. Increase in weight of total accessory reproductive organs (14, 15).

The method of assay[1] was as follows:—The test animals were of the Wistar strain (Edinburgh), and of the Leila strain (Stockholm), respectively. They varied in age from 19–23 days and in weight from 35–45 Gm. The animals were injected subcutaneously once per day on three consecutive days at approximately 24-hour intervals. They were killed on the fourth day and the accessory reproductive organs carefully dissected out from the surrounding structures and fixed in Bouin's solution for twenty-four hours. On the fifth day the organs were freed from fat, dried between pieces of filter paper and weighed on a torsion balance. The results were expressed as mg.organ weight/100 Gm. rat weight (Edinburgh) and as mg.organ weight/rat (Stockholm), respectively.

(b) In *hypophysectomized immature male rats* the indices of response were:

1. Increase in ventral prostatic weight (16).
2. Increase in total prostatic weight.
3. Increase in seminal-vesicle weight.

The technique was similar to that described by Loraine and Brown (17) for the assay of Human Menopausal Gonadotropin (H.M.G.). The animals were hypophysectomized at a body weight of 40–50 Gm. Injections were commenced on the fifth day after hypophysectomy, one subcutaneous injection being given on each of four consecutive days. The animals were killed on the fifth day, the prostate and seminal vesicles fixed in Bouin's solution for twenty-four hours, and weighed on the following day.

(c) In *intact immature female rats* assays depended on:

1. Increase in uterine weight (2, 18).
2. Increase in ovarian weight (19, 20).

In the assay method depending on the *uterine weight* (Stockholm) rats varying in weight from 36 to 44 Gm. and in age from 20 to 23 days were employed. Injections were made subcutaneously once per day for three days. On the fourth day the animals were killed, the uterus removed, dissected free from fat, dried on filter paper and weighed on a torsion balance. In the procedure used in Edinburgh the dosage schedule and time of sacrifice were identical with those used in Stockholm, but the uteri were fixed in Bouin's solution for twenty-four hours before weighing. The uterine weight was not adjusted for differences in body weight.

In the *ovarian-weight* method the age and weight of the rats were the same as in the uterine-weight method. The animals were injected subcutaneously on each of four consecutive days. On the fifth day, they were killed, the ovaries carefully dissected out from surrounding fat and connective tissue, and weighed on a torsion balance.

C. *Design of assays*

In all, 35 assays were performed; 19 of the 35 were conducted on serum and 16 on plasma. In 32 instances the design was a symmetrical four-point assay with two dose levels of the standard (S) and unknown (U) preparations, respectively, and with equal spacing of log doses. Equal numbers of animals were used at each dose level of S and U. In 15 of the 32 four-point assays a completely randomized block design was employed,

[1] Unless specially indicated, assay conditions in the two laboratories were identical.

with an equal number of rats at all dose levels and in all litters, one rat from each litter being assigned to each of the four doses (litter-mate control). The technique is described elsewhere in detail (15). In 3 assays involving the use of hypophysectomized rats a six-point design (3 doses of S versus 3 doses of U) was employed. Validity tests were performed in each assay, following the technique described by Finney (21) and Gaddum (22, 23). No significant deviation from parallelism occurred in any assay. The log dose interval, *i.e.*, the difference between the logarithms of two neighboring doses was equal to $\log_{10}^2$ (0.301) in all the assays.

In intact immature male rats the total dosages of the international standard preparation administered per animal over the three-day injection period were 2.0 and 4.0 I.U. (Edinburgh) and 1.5 and 3.0 I.U. (Stockholm). In hypophysectomized rats the total dosages were 2.0 and 4.0 I.U. when the four-point design was employed, and 1.0, 2.0 and 4.0 I.U. when the six-point design was used. In assays involving uterine weight the total amount administered was 0.8 and 1.6 I.U. (Edinburgh) and 0.6 and 1.2 I.U. (Stockholm), respectively. When serum was injected (total doses of 1.0 ml. and 3.0 ml.) without the addition of H.C.G., there was no increase in uterine weight over control animals injected with saline. In the ovarian-weight method, in order to obtain a significant response, it was necessary to administer total dosages of 18.0 and 36.0 I.U.

D. *Indices of precision*

For comparison of alternative assay techniques, or of the same technique in the two laboratories, the following indices of precision were estimated:

(a) *Gaddum's* λ, which is equal to the standard deviation of the logarithms of the individual effective doses (24, 26). This quantity is independent of the units of measurement and of the experimental design. For assays depending on graded responses, the ratio of the standard deviation of the response to the slope of the regression line, *i.e.*, s/b, gives an estimate of λ.

(b) *Woolf's L*, which is the reciprocal of λ (25). It has been claimed recently by Woolf that in assays depending on graded responses this quantity should be used instead of λ, since when the total number of observations in each experiment is large, the distribution of L is approximately normal with variance $1/S(x-\bar{x})^2$. Gaddum (23) has suggested that in addition to other indices of precision, such as λ or g, different assay techniques should be compared in terms of their mean value of L.

In the present paper the value of λ for each assay technique is estimated from the expression $\bar{s}/\bar{b}$ where $\bar{s}$ is the square root of the weighted error mean square computed from all the experiments with this technique; $\bar{b}=S(wb)/S(w)$ and $w=1/V(b)$ in each assay (15). The results are shown in Table 4.

(c) *Finney's g*, which is an estimate of the significance of the regression coefficient, and gives an indication of the certainty with which the regression coefficient (b) is estimated (21, 27). Finney's *g*-criterion is defined as

$$g = V(b) \cdot t^2/b^2$$

where t is the 0.05 probability deviate with the number of degrees of freedom for the error mean square and V(b), the variance of b, s^2/S_{xx}; (s^2 is the error mean square and S_{xx} indicates the sum of squares of deviations of doses, *i.e.*, $S(x-\bar{x})^2$). For an accurate assay *g* is 0.1, or less; a value of unity or more shows, on the other hand, that there is no real regression and that the assay is therefore invalid.

E. *Calculation of the relative potency and of the limits of error*

The estimate of the relative potency (R) was calculated by using the log ratio of po-

tencies (M), estimated with Gaddum's (24) equation as

$$M = \bar{x}_S - \bar{x}_U - \frac{\bar{y}_S - \bar{y}_U}{b}$$

where $\bar{x}_S$ and $\bar{x}_U$ indicate the mean dose, and $\bar{y}_S$ and $\bar{y}_U$ the mean response of the standard and unknown preparations respectively, and b is the average regression coefficient calculated by pooling the data for standard and unknown. By preliminary pilot investigations the potency of U could be predicted with reasonable accuracy in all assays, and dosages were chosen so that the unknown preparation would give approximately the same average effect as the international standard. Fiducial limits of error were calculated at a probability of 0.95 according to the formula given by Finney (21), the limits to $(M - x_S + x_U)$ being equal to

$$\left[M - \bar{x}_S + \bar{x}_U \pm \frac{ts}{b}\left\{(1-g)\left(\frac{1}{N_S} + \frac{1}{N_U}\right) + \frac{(M - \bar{x}_S + \bar{x}_U)^2}{\Sigma S_{xx}}\right\}^{1/2}\right] \div (1-g)$$

where N_S and N_U indicate the total number of animals on standard and unknown preparations respectively, the symbol $\pm$ indicates the two alternate operations (addition and subtraction respectively) for the upper and lower fiducial limits of error, and ΣS_{xx} stands for summation extended over each reading. In all cases this exact formula was used for the calculation of fiducial limits, even in those assays in which g was less than 0.1. Thus, in the assays reported, the actual potency can be assumed to lie within the fiducial limits 95 times out of 100.

RESULTS

Validity of H.C.G. assays conducted on serum or plasma

In Table 1 are shown the results obtained in 21 assays, using intact immature male rats in which the activity of H.C.G. dissolved in serum or plasma (U) was compared with that of H.C.G. dissolved in water or saline (S), using as indices of response (i) prostatic weight, (ii) seminal-vesicle weight, and (iii) weight of total accessory reproductive organs. Without taking into account differences in the amount of serum or plasma administered or variation in the precision of individual assays, the mean percentage potency of U for the prostate was 106, for the seminal vesicles 114, and for the total accessory reproductive organs 109. It will be noted that in 20 out of 21 assays the figure of 100 per cent was within the fiducial range. It therefore seems justifiable to conclude that the potency of U is identical with that of S.

The results obtained in hypophysectomized immature male rats are shown in Table 2. In 4 assays involving the prostate (ventral lobe and total gland) the mean percentage potency of U was 117, and in 2 assays using seminal vesicles, 121.5. In all 6 experiments the figure of 100 per cent was within the fiducial range. In 26 out of 27 assays conducted on immature male rats a reasonably high degree of accuracy was obtained and the value of 100 per cent could safely be proposed for the true potency of U. It therefore appears justifiable to conclude that the effect of H.C.G. on the various accessory reproductive organs of intact or hypophysectomized immature

TABLE 1. COMPARISON OF THE ACTIVITY OF H.C.G. DISSOLVED IN SERUM OR PLASMA (U) WITH THAT OF H.C.G. DISSOLVED IN WATER (S) IN INTACT IMMATURE MALE RATS

End-point of assay	Assay material	Tot. vol. solvent/ rat (ml.)	No. of rats/ assay	Potency of U (%)	Fiducial limits (P=0.95)	Indices of precision			Laboratory
						λ	L	*g*	
Wt. of prostate	serum	3.0	24	109	90–135	0.076	10.54	0.095	S*
	serum	1.0	16	105	88–128	0.075	13.37	0.080	E†
	serum	1.0	20	95	81–110	0.067	14.90	0.050	E
	plasma	3.0	20	111	84–151	0.119	8.40	0.150	S
	plasma	3.0	20	112	89–114	0.101	9.90	0.160	S
	plasma	1.0	20	123	92–177	0.137	7.32	0.163	E
	plasma	1.0	16	87	65–111	0.100	10.08	0.118	E
Wt. of sem. vesicles	serum	3.0	24	94	77–150	0.144	6.94	0.179	S
	serum	1.0	16	104	79–138	0.102	9.77	0.126	E
	serum	1.0	20	106	83–138	0.115	8.80	0.128	E
	plasma	3.0	20	115	85–167	0.131	7.62	0.183	S
	plasma	3.0	20	138	102–214	0.131	7.63	0.184	S
	plasma	1.0	20	113	67–214	0.211	4.75	0.390	E
	plasma	1.0	16	128	70–374	0.204	4.81	0.500	E
Wt. of total access. reprod. organs	serum	3.0	24	105	85–133	0.104	9.66	0.094	S
	serum	1.0	16	107	93–126	0.074	13.52	0.065	E
	serum	1.0	20	103	88–120	0.071	14.08	0.044	E
	plasma	3.0	20	118	100–140	0.070	14.21	0.052	S
	plasma	3.0	20	118	98–145	0.083	12.05	0.072	S
	plasma	1.0	20	108	85–137	0.095	10.50	0.080	E
	plasma	1.0	16	110	68–191	0.166	6.03	0.330	E

* S = Stockholm. In all assays performed in Stockholm, litter-mate control was employed.

† E = Edinburgh.

male rats is not significantly increased when the hormone is dissolved in human serum or plasma instead of water. Accordingly clinical bioassays of H.C.G. in untreated serum or plasma are valid, if the male accessory reproductive organs are used as indices of response.

Results obtained in intact immature female rats are shown in Table 3. In 6 assays depending on the increase in uterine weight, the mean percentage potency of U was 188. It will be noted that in none of these 6 assays was it possible to propose 100 per cent as a possible value for the true potency. Thus it is apparent that estimates by the uterine-weight method of

TABLE 2. COMPARISON OF THE ACTIVITY OF H.C.G. DISSOLVED IN SERUM (U) WITH THAT OF H.C.G. DISSOLVED IN WATER (S) IN HYPOPHYSECTOMIZED IMMATURE MALE RATS (EDINBURGH)

End-point of assay	Assay material	Assay design	Tot. vol. solvent /rat (ml.)	No. of rats/ assay	Potency of U (%)	Fiducial limits (P = 0.95)	Indices of precision		
							λ	L	*g*
Ventral prostatic wt.	serum	4-point	1.0	18	114	80–179	0.147	6.79	0.227
Ventral prostatic wt.	serum	6-point	1.0	20	116	70–206	0.200	5.04	0.166
Total prostatic wt.	serum	4-point	1.0	18	111	71–178	0.165	6.08	0.270
Total prostatic wt.	serum	6-point	1.0	20	127	72–257	0.240	4.14	0.232
Seminal-vesicle wt.	serum	4-point	1.0	18	127	95–182	0.116	8.58	0.144
Seminal-vesicle wt.	serum	6-point	1.0	20	116	82–166	0.150	6.68	0.093

TABLE 3. COMPARISON OF THE ACTIVITY OF H.C.G. DISSOLVED IN SERUM OR PLASMA (U) WITH THAT OF H.C.G. DISSOLVED IN WATER (S) IN INTACT IMMATURE FEMALE RATS

End-point of assay	Assay material	Tot. vol. solvent /rat (ml.)	No. of rats/ assay	Potency of U (%)	Fiducial limits (P = 0.95)	Indices of precision			Laboratory
						λ	L	*g*	
Uterine wt.	serum	3.0	24	159	136–184	0.068	14.71	0.048	S*
Uterine wt.	serum	3.0	24	208	179–244	0.073	13.75	0.047	S
Uterine wt.	serum	1.0	16	169	120–226	0.110	9.15	0.144	E†
Uterine wt.	serum	1.0	16	229	200–264	0.050	19.87	0.031	E
Uterine wt.	plasma	3.0	28	180	154–208	0.083	12.02	0.050	S
Uterine wt.	plasma	3.0	28	185	152–223	0.097	10.33	0.072	S
Ovarian wt.	plasma	4.0	24	140	87–390	0.232	4.30	0.455	S
Ovarian wt.	plasma	4.0	20	145	86–602	0.229	4.36	0.551	S

* S = Stockholm. In all assays performed in Stockholm, litter-mate control was employed.

† E = Edinburgh.

the serum concentration of H.C.G. give an overestimate of activity, and that results obtained in this way must be considered to be invalid. In the two assays depending on the increase in ovarian weight the mean percentage potency of U was 143. However, the accuracy of this method in the hands of the present investigators was so low that it was not possible to assess whether plasma had any significant effect in increasing or decreasing the biologic activity of H.C.G. It is, however, conceivable that with a different strain of animals or under different experimental conditions results of quantitative significance might be obtained with the ovarian-weight test.

Comparison of precision of assay methods for H.C.G.

The results are shown in Table 4. The indices of precision obtained, using various end-points of assay, confirm previous observations (12, 14, 15) that for the assay of H.C.G. in intact immature rats, the test depending on seminal-vesicle weight is less accurate than those involving prostatic weight or weight of the total accessory reproductive organs. It is also apparent that the prostatic-weight test is less accurate in hypophysectomized than in intact rats. The uterine-weight method, in the experience of the authors, has a high degree of precision but is unsuitable for the assay of H.C.G. in serum or plasma for the reasons given. The ovarian-weight test is both inaccurate and insensitive and cannot therefore be recommended for the quantitative assay of H.C.G. in body fluids.

DISCUSSION

The present studies indicate that the effect of H.C.G. on the weight of the accessory reproductive organs of immature male rats is not influenced

TABLE 4. ESTIMATES OF ERRORS OF TESTS FOR H.C.G.

Type of rat	Response	No. of assays	Weighted mean λ $-(\lambda_w)$	Weighted mean L $-(L_w)$
Intact immature	Prostatic wt.	7	0.108	9.25
	Seminal-vesicle wt.	7	0.143	7.00
	Wt. of total accessory reproductive organs	7	0.109	9.18
Hypophysectomized immature	Prostatic wt. (ventral lobe and total gland)	4	0.184	5.47
	Seminal-vesicle wt.	2	0.128	7.80
Intact immature	Uterine wt.	6	0.086	11.68
	Ovarian wt.	2	0.240	4.16

appreciably when the hormone is dissolved in human serum or plasma instead of saline. This confirms a previous observation (4) that the serum proteins do not interfere with the assay which depends on prostatic weight.

The results obtained by the uterine-weight method in intact immature rats are in essential agreement with the general conclusions of Leach *et al.* (11) as to the "augmentation" of the biologic activity of H.C.G. when administered in serum or plasma instead of saline. It would, therefore, appear that bioassays of H.C.G. in serum or plasma by the uterine-weight method as recommended by Jones *et al.* (1) and by Dorfman and Rubin (2) will most probably give an overestimate of the real potency and will thus yield erroneously high serum values.

On the other hand, the results in this paper are at variance with those reported by Leach *et al.* (11) as far as the quantitative aspects of this "augmentation" are concerned. Whereas Maddock and Leach (9) reported a five fold to tenfold increase in potency when H.C.G. is mixed with plasma, the present experiments indicate only an approximate doubling of potency. Whether this discrepancy can be ascribed to differences in the schedule of injections or to differences in the strains of the animals cannot be decided at present. Since the error of the assays conducted by Leach *et al.* (11) cannot be calculated from the experimental data presented, it is difficult to assess the quantitative significance of their findings. Accordingly their suggestion that estimates of H.C.G. in pregnancy serum previously reported in the literature using the uterine-weight method are in error by a factor of 10, would appear to be unjustified.

It is of considerable interest that in certain types of assay an underestimate of potency has been observed when H.C.G. was dissolved in human serum. Thus Salvatierra and Torres (28), using the assay method based on the expulsion of spermatozoa in *Rana esculenta*, reported that three times as much H.C.G. was required to produce a positive response when the hormone was dissolved in serum instead of in saline. Similarly Albert and Berkson (7) noted that the response obtained with undiluted serum in the ovarian hyperemia test in rats was only one third of that obtained with the standard H.C.G. in saline. They recommend, therefore, that the serum be diluted 1:10. However, the possibility that the ovarian hyperemia reaction gives an overestimate when the assay is conducted on diluted but untreated serum should also be considered. Such an assumption would seem to be supported by the discrepancy in the figures for renal clearance of H.C.G. reported by Gastineau, Albert and Randall (3) on the one hand, and by Loraine (4) on the other. Using the ovarian hyperemia reaction, the former group of workers found that the serum concentration of H.C.G. was approximately three times that found in the urine, whereas Loraine (4), employing the prostatic-weight method, reported that the concentra-

tion of H.C.G. in the serum was approximately equal to that in urine. In view of the relatively low accuracy of the ovarian hyperemia method as described by Albert and Berkson (7), it is difficult to exclude the possibility that the figures obtained by this method for the concentration of H.C.G. in pregnancy serum may be erroneously high.

When the international standard for H.C.G. was adopted in 1939, it was emphasised that the international unit should be used for recording the activities of all gonadotropic preparations of human pregnancy urine, *but only of such*. The data presented in the present paper show that under certain circumstances it is not justifiable to assay serum samples of H.C.G. against the standard preparation. Difficulties of a similar nature will probably be encountered when body fluids or organ extracts containing adrenocorticotropic, thyrotropic or growth-promoting activity are assayed against the corresponding standards prepared from anterior pituitary tissue. Accordingly it is essential to establish by statistical methods whether or not such assays are valid before employing them in the clinical field for diagnostic and metabolic studies.

SUMMARY

The biologic activity of human chorionic gonadotropin (H.C.G.) dissolved in serum or plasma has been compared with that of H.C.G. dissolved in saline, using a variety of bioassay methods in intact and hypophysectomized rats. Assays were conducted in two independent laboratories.

1. The effect of H.C.G. on the various accessory reproductive organs of intact or hypophysectomized immature male rats was not increased significantly when the hormone was dissolved in serum or plasma instead of saline. Accordingly, clinical bioassays of H.C.G. in untreated serum or plasma are valid if the prostatic weight, seminal-vesicle weight, or the weight of the total accessory reproductive organs are used as indices of response.

2. Under the experimental conditions used, the effect of H.C.G. in the uterine-weight test in rats was increased approximately twofold when the hormone was dissolved in serum or plasma instead of saline. This method is not suitable for the assay of H.C.G. in untreated serum or plasma.

3. Assays depending on the ovarian-weight test were so inaccurate that it was not possible to assess whether plasma has any significant effect in increasing or decreasing the biologic activity of H.C.G.

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THE WEIGHT AND EXTRACTABLE INSULIN OF THE PANCREAS IN ADULT NONDIABETIC MAN

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INTRODUCTION

RECENTLY Jorpes and Rastgeldi (1) have published values for the *concentration* of insulin in human pancreas, the average of which is significantly higher[1] than that reported by Wrenshall, Bogoch and Ritchie (2). In both cases, ethyl alcohol-HCl extraction procedures were employed.

The foregoing difference is more apparent than real, since comparison of both sets of data has revealed that, when the extractable insulin is expressed as units *per whole pancreas* for adult subjects, the average values in the two series no longer differ significantly. Analysis of our data for adult nondiabetic subjects has indicated that the concentration of extractable insulin in the pancreas is inversely correlated with the total fresh weight of the gland. The significance of this correlation will be considered in the light of the related finding that the total fresh weight of pancreas varies greatly in adult human subjects.

MATERIALS AND METHODS

The data employed in the correlations which follow represent those reported by Wrenshall, Bogoch and Ritchie (2) augmented by more recently accumulated values, obtained by use of the same standardized procedures. Nondiabetic subjects who were known to have received treatment with ACTH, cortisone or insulin were eliminated from the series, since these factors have been found to be associated with changes in the extractable insulin of the pancreas (3) and in islet volume (4). All subjects who were more than 25 per cent above or below standard[2] body weight for their sex, age

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[1] For 52 hospitalized nondiabetic adult subjects, we found the average concentration of extractable insulin in the pancreas to be 2.37 ± 0.14 units per gram of pancreas (2), as compared with Jorpes and Rastgeldi's value of 3.66 ± 0.12 units per gram of pancreas (1). The difference is highly significant in a statistical sense.

[2] Standard values for the calculation of percentages of overweight and underweight were taken from Volume I of Medico-Actuarial Mortality Investigation, published by

and height were excluded, since such marked deviations were interpreted as representing the results of gross overnutrition or undernutrition—factors which have been observed to be associated with changes in the insulin content of the pancreas (2, 5). The resulting series consisted of 48 adult male subjects and 27 adult female subjects.

RESULTS AND OBSERVATIONS

The frequency distributions for wet weight of pancreas[3] for 307 male and 220 female diabetic subjects are shown in Figure 1. The average value for the female subjects (82 grams) is smaller than that for male subjects (103 grams) in accordance with the sex difference in body weight. The range over which pancreas weight varied in adult nondiabetic males (25–275 grams) and females (35–220 grams) is large in each case.

A scatter diagram, in which the concentration of extractable insulin in the pancreas is plotted against the total fresh weight of the pancreas for adult nondiabetic human subjects, is shown in Figure 2. Equations of hyperbolae of best fit and coefficients of correlation are shown in Table 1 for each sex, as well as for the entire set of data; they are compared with similar measures determined from the data of Scott and Fisher (6).

It is apparent from each of these statistical analyses that the *concentration* of extractable insulin in nondiabetic human pancreas, expressed as units of insulin per gram of pancreas, shows a highly significant degree of inverse correlation with the total fresh weight of the pancreas. The subjects of Scott and Fisher were in apparent good health before death, and hence represent a more homogeneous group than ours, most of whom died following a period of hospitalization for various conditions. It is, therefore, not surprising to find a closer correlation between concentration of insulin in the pancreas and the reciprocal of pancreas weight in their series than in our less homogeneous one.

No such correlation is found for those adult subjects of Jorpes and Rast-

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the Association of Life Insurance Medical Directors and the Actuarial Association of America, New York, 1913. Standard subjects of the 1912 series were weighed while wearing "usual indoor clothing" with shoes removed. The subjects included in the current series, whose weights are compared with such standards, were weighed at autopsy without clothing. It is, therefore, clear that all of the percentage overweight figures cited in the text are too low.

[3] The measurements cited were taken from protocols describing the findings in routine autopsies performed by various staff members of the Department of Pathology, University of Toronto. In addition to the subjects of the present survey on the insulin extractable from the pancreas, the study included others who were referred for autopsy from the Toronto General Hospital during the two years 1930 and 1940. All adult nondiabetic subjects, with the exception of those dying with cancer involving the pancreas, were included.

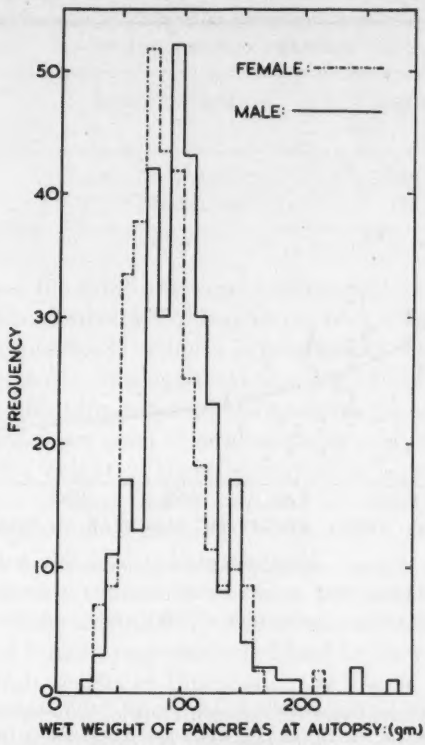

FIG. 1. Frequency distributions for weight of pancreas of adult nondiabetic human subjects at autopsy. The histograms for the 307 male and 220 female subjects are made to enclose equal areas. Data of the Department of Pathology, University of Toronto, Canada.

geldi who were studied individually. Nevertheless the average *total* insulin extracted from the pancreas in their subjects is found to show no statistically significant difference from the average values for the adult nondiabetic human subjects of our published series (Table 2).[4]

DISCUSSION

An obvious factor contributing to the inverse correlations of Table 1 is lack of uniformity in the trimming of adherent material from a pancreas prior to its weighing. If, for example, some adjacent splenic tissue were in-

[4] It appears unlikely that variations in the length of time between death and extraction of insulin from the pancreas could account for the observed difference. No correlation between the amount of insulin extracted from human pancreas and the number of hours post mortem of autopsy (and of extraction) was observed in either series (1, 2).

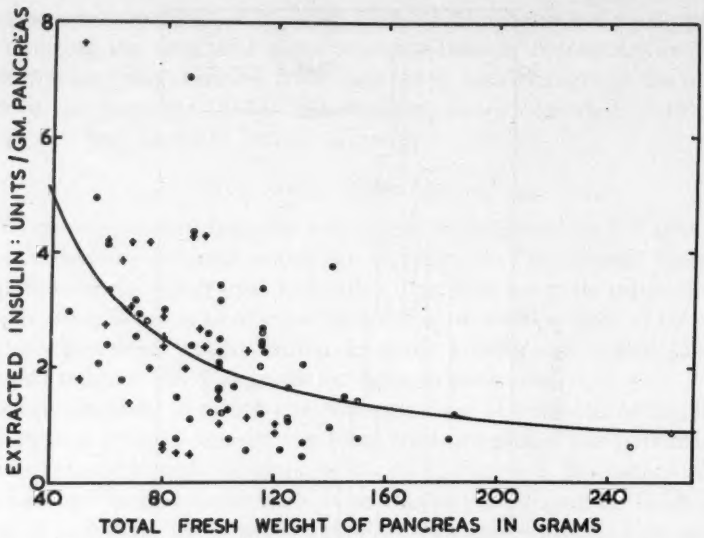

FIG. 2. Correlation diagram associating the concentration of extractable insulin in the pancreas with the total fresh weight of the pancreas in adult nondiabetic human subjects. Female subjects (+); male subjects: (●); male subjects of Scott and Fisher (1938): (○).

TABLE 1. DEGREES OF LINEAR CORRELATION, ASSOCIATING AMOUNT OF EXTRACTABLE INSULIN PER GRAM OF PANCREAS (I), WITH THE RECIPROCAL OF THE PANCREAS WEIGHT (P) MEASURED IN GRAMS FOR ADULT NONDIABETIC HUMAN SUBJECTS

Sex	No. of subjects	Straight line of best fit	Coefficient of linear correlation ± S.E.*
Scott & Fisher (1938):			
Male	9	$I = -0.41 + \frac{253}{P}$	0.79 ± 0.27
Present series:			
Male	48	$I = -0.47 + \frac{163}{P}$	0.44 ± 0.12
Female	27	$I = -0.75 + \frac{252}{P}$	0.64 ± 0.13
Male & female	75	$I = +0.14 + \frac{189}{P}$	0.45 ± 0.09

* By *t*-test, all of these coefficients of correlation are significant at the $p = 0.001$ level.

TABLE 2. EXTRACTABLE INSULIN AS UNITS PER WHOLE PANCREAS ± S.E. IN ADULT HUMAN SUBJECTS

	Jorpes & Rastgeldi (1)	Wrenshall, Bogoch & Ritchie (2)
Male subjects	246 ± 21 (7 subjects)	216 ± 14 (48 subjects)
Female subjects	189 ± 19 (4 subjects)	183 ± 17 (27 subjects)

cluded as pancreas, the total wet weight designated as pancreas would be increased and the concentration of insulin per unit weight of extracted tissue would be correspondingly reduced in magnitude. Although the example chosen is an extreme one, it is apparent that the extent to which non-islet tissue is dissected out of the pancreas will serve on the one hand to increase the amount of insulin per gram of residual tissue and, on the other, to decrease the total wet weight of tissue described as pancreas. In our series we made a practice of dissecting off *adherent* fat and other encapsulating tissue associated with human pancreas, but not of removing blood vessels or fatty tissue lying within the pancreas itself.

An important factor influencing the total wet weight of pancreas, and one which is less easily eliminated, is its fat content. In the experience of Balō (7), the fat of human pancreas was found to vary between 2 and 74 per cent of the fresh weight of this tissue, the degree of variation being particularly pronounced in older subjects. In most instances the fat of the pancreas is distributed interstitially throughout the volume of the tissue, so that to dissect it out completely would represent an impossible task. In 2 subjects of the present series, samples of fat dissected out of human pancreas were extracted for insulin, but none was detected.

The wide range in the fat content of human pancreas raises an interesting point. If the amount of insulin extracted per gram of adult human pancreas remains within the narrow range of values (between 3 and 4 units per gram, average 3.66 ± 0.12) observed by Jorpes and Rastgeldi, then the concentration of insulin per gram of *fat-free* pancreas must be extremely variable.

The existence of a very large range of variation in the total wet weight of adult human pancreas (Fig. 1) raises another point. This large variation,[5] together with the finding of Jorpes and Rastgeldi that the insulin extracted per gram of pancreas is effectively constant in adult human subjects, would lead to the conclusion that the amount of insulin per whole pancreas is highly variable in such subjects. The finding of an inverse cor-

[5] The sixfold to elevenfold range of variation is based on the data of Figure 1. A sixfold variation was found in the range for the subjects in whom pancreatic insulin determinations were made (Fig. 2).

relation between extractable insulin per gram of pancreas and total pancreas weight in comparable subjects, reported in this paper, indicates that the reverse is nearer to the truth. In other words, the foregoing correlation indicates that the *total* reserve of pancreatic insulin is less variable than the amount of insulin per unit weight of tissue, a situation which might be anticipated on purely physiologic grounds.

Although the results in Table 2 show no *statistically* significant differences between the average pancreatic reserves of insulin in adult subjects, the values of Jorpes and Rastgeldi are, in each case, slightly higher (13 per cent, 3 per cent) than those of Wrenshall, Bogoch and Ritchie. The alcoholic extracts of insulin made by Jorpes and Rastgeldi were assayed without further processing. Our acid alcoholic extracts were rendered alkaline and then filtered; the insulin was precipitated out by the addition of alcohol and ether, and the precipitates were finally dissolved in acid saline prior to assay. It is almost certain that small losses of extracted insulin occurred during the precipitations.

Related to the foregoing comparison is a question of terminology. The term "insulin content of pancreas" has been frequently used to describe the amount of insulin extracted from the pancreas by a stated procedure. This term implies the unproven assumption that *all* of the insulin has been extracted from the pancreas by the procedure employed. A study of the history of insulin extraction procedures indicates that increases in the insulin extractable from the pancreas occasionally accompany changes in extraction technique. Hence, we have proposed that the term "insulin content of pancreas" should be employed only when accompanied by evidence that it is what it purports to be (2). Alternatively, having determined a value, x, for the insulin extracted from a pancreas, one might safely state that the insulin content of that pancreas was equal to, or greater than x.

SUMMARY

The existence of an inverse correlation between the amount of extractable insulin per unit weight of pancreas and the total weight of the pancreas in adult human subjects has been demonstrated. Such a correlation, combined with the finding that the total weight of the pancreas is widely variable in man, indicates that in man the extractable insulin per gram of pancreas cannot be considered to represent a characteristic constant.

Acknowledgments

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THE RELATIONSHIP BETWEEN METABOLIC ACTIVITY AND IODIDE-CONCENTRATING CAPACITY OF SURVIVING THYROID SLICES*†

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IN 1944, Schachner, Franklin and Chaikoff demonstrated that the concentration of inorganic I^{131} by surviving slices of sheep thyroid tissue could be dissociated from organic binding by sulfanilamide or azide (1). They postulated the existence within the thyroid of "a mechanism for concentrating iodine that does not depend upon the conversion of inorganic iodide to thyroxine and diiodotryosine." Subsequent workers showed that this concentrated iodine is dialyzable, ultrafilterable, nonprecipitable, and potentiometrically as well as chromatographically indistinguishable from ionic iodide (2, 3). Measurements of thyroid/serum concentration ratios for inorganic iodide have been made in the rat (2–4), mouse (5), chick-embryo (6), and man (7, 8). *In vivo*, these gradients may be enhanced by thyroid-stimulating hormone and depressed by hypophysectomy (4, 5, 9, 10, 11) or prolonged administration of thyroxine (9, 10). Elevation of the plasma iodide level may saturate the mechanism (2, 7, 11) and thus depress concentration ratios, although the absolute content of inorganic iodide within the gland is augmented (2, 11). Contrariwise, monovalent anions such as thiocyanate (2, 12), perchlorate, and nitrate reduce both the thyroid/serum ratio and the thyroidal stores of iodide by interfering with glandular retention of ionic iodide (13).

In none of the published reports has there been a definition of the mech-

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anism whereby inorganic iodide enters the thyroid and concentration differentials are established. Reviewers variously have suggested that the process is mediated by binding to intrathyroidal protein, or by active enzymatic transfer, or by a combination of both mechanisms (14, 15). The present studies were undertaken to evaluate these possibilities by examining the accumulation of inorganic iodide *in vitro* over a wider range of experimental conditions than would be possible in the intact animal. Concentration of inorganic I^{131} by sheep thyroid slices was examined, and related to simultaneous estimation of cellular oxygen consumption in the Warburg apparatus. Organic binding of iodine was eliminated by the inclusion of 1-methyl-2-mercaptoimidazole in the suspending medium. The works of Forster and Taggart (16), Cross and Taggart (17), and Mudge (18, 19) on transport mechanisms in isolated renal tubules and slices of rabbit kidney cortex were used as prototypes for many of the experiments.

METHODS

Fresh intact thyroid glands were obtained from sheep or lambs which had been sacrificed by exsanguination in the local abattoir (20). Tissues were partially freed of fat and fascial investments and were wrapped in cellophane bags, placed on cracked ice, and transported to the laboratory in Dewar flasks. Following variable storage for one to three hours at 4° C., the glands were removed from cellophane and placed in Petri dishes containing chilled 0.15 M sodium chloride. A rectangular block was excised from the central core of each lobe, thereby removing all fascial, capsular and subcapsular material. Slices of thyroid tissue of 0.3–0.4 mm. thickness were prepared with a Stadie-Riggs microtome. In most experiments, all slices were derived from a single lobe; occasionally, paired lobes from a single thyroid were pooled to provide adequate amounts of tissue.

Slices were briefly blotted on filter paper and weighed on a torsion balance. A sample of tissue weighing approximately 150–300 mg. was put into 3.0 ml. of suspending medium contained within the main compartment of Warburg flasks, which were kept chilled on cracked ice. In any single experiment the tissue content of individual flasks did not differ by more than 20 per cent.

Krebs-Ringer-Phosphate (21) (KRP) buffered at pH 7.4 and containing 0.7–1.0 μc. of I^{131} per ml. was employed as suspending medium. Organic binding of iodine was inhibited by the routine incorporation of 1-methyl-2-mercaptoimidazole (Tapazole) at a final concentration of 1×10^{-3} M (KRP-Tap.10^{-3})[1]. No stable iodide was added to the medium; however, the reagent contamination in the preparation of Krebs-Ringer solutions has been estimated at 0.1 μg. of I^{127} per ml. (22).

Flasks were placed in a thermostatically controlled water bath at 38° C. and shaken at 104 cycles per minute after the addition of 0.2 ml. of 10 per cent potassium hydroxide to the center well. Gas spaces were filled for five minutes with 100 per cent oxygen. Following fifteen to twenty-five minutes of temperature equilibration, oxygen consumption was measured at ten-minute intervals for one hour by direct manometry (21). Oxygen consumption (QO_2) was derived for each flask as cu. mm. of O_2 per hour per mg. of initial

[1] In "tip-in" experiments, 0.3 ml. of KRP-Tap.10^{-3} was added to the side arm, and 2.7 ml. placed in the main compartment. Radioactivity was confined to either side arm or main compartment in accordance with experimental objectives.

wet weight. In many experiments, dry weight/wet weight ratios were obtained by drying representative slices to constant weight at 105° C.

Following incubation, slices were quickly removed from the flasks, blotted on filter paper, and weighed on the torsion balance. Suspending solutions were centrifuged immediately and residual radioactivity was measured from 1.0-ml. aliquots. In early experiments, radioactivity within the slices was partitioned into inorganic and organic moieties by homogenization-extraction in ground-glass homogenizers containing 2.0 ml. of 10 per cent trichloracetic acid (TCA). Homogenates were centrifuged and successively washed with 5.0-ml. portions of 2.5 per cent TCA and distilled water. Washings were alkalinized and pooled into a TCA-soluble fraction which was considered to contain thyroidal inorganic iodide. The acid-insoluble residues containing protein-bound I^{131} were digested in 2.0 ml. of 2N sodium hydroxide. In 118 experiments with KRP-Tap.10^{-3}, acid-insoluble radioactivity did not exceed 0.1 per cent of the administered counts per 100 mg. of tissue. Therefore, in later experiments, slices were digested directly in 2 ml. of 2N sodium hydroxide, and all recovered I^{131} was included in the inorganic moiety. Planchets were prepared with 1.0-ml. aliquots of diluted medium, inorganic fractions, and organic fractions. Mass did not exceed 10 mg. Radioactivity was measured with a mica end-window Geiger-Mueller tube; sufficient counts were observed to reduce the probable error of the measurement to less than 1 per cent. Counts in each fraction were related to the final wet weight of the tissue. Concentration gradients (slice/medium ratios; S/M I^{131}) were derived as:

$$\frac{\text{counts in inorganic fraction/Gm. of tissue}}{\text{counts/ml. of medium}}.$$

This calculation provides a more valid estimate of the capacity to concentrate inorganic iodide than measurements based on the percentile accumulation of the administered radioactivity.

If

y = the percentage of the radioactivity which is concentrated per 100 mg. of tissue

and

$\frac{100 - y}{3}$ = the percentage of the radioactivity which remains within each 1.0-ml. portion of suspending medium,

then

$$S/M\ I^{131} = \frac{10y}{(100 - y)/3} = \frac{30y}{100 - y} = X$$

and

$$y = \frac{100X}{30 + X}.$$

S/M I^{131} may be plotted as a function of the percentile accumulation of radioactivity (Fig. 1). The relationship approximates linearity when concentration differentials are low. However, when cellular avidity for iodide is enhanced, the medium becomes depleted of radioactivity and profound changes in S/M ratios will result in only minor variation in the percentile accumulation. When slices from a single gland are removed from experimental flasks after uniform incubation, S/M gradients serve as a reproducible parameter. S/M ratios remain constant despite gross differences in percentile accumula-

TABLE 1. EFFECT OF VARIATION IN SUSPENDING VOLUME AND TISSUE MASS UPON S/M^{131}

Exp. No.	Total recovery (% admin'd counts)	Suspending vol.* (ml.)	Tissue wt.† (mg.)	QO_2	S/M I^{131}	% Recovered I^{131} / 100 mg. tissue
192	88.1	2.5	223.0	0.66	51.9	39.0
	88.5	3.0	181.0	0.66	49.4	43.9
	84.6	3.5	221.0	0.70	50.6	35.2
164	89.2	3.0	109.0	0.74	111.1	84.7
	90.8	3.0	138.0	0.76	112.7	69.9
	86.9	3.0	204.5	0.75	119.8	52.9
	94.0	3.0	307.5	0.77	111.8	34.7

* KRP-Tap.10^{-3}.

† Initial wet weight of slices.

tion produced by varying the volume of suspending medium or the mass of incubated tissue (Table 1).

RESULTS

I. *General experimental technique*

The use of abattoir products entails technical variation in transportation and storage. In addition, previous diet, age of donor animals, and seasonal exposure[2] cannot be controlled. Thus, S/M ratios and oxygen consumption varied widely in different experiments. However, within any single experiment, adequate uniformity could be achieved by filling both control and experimental flasks with slices from the same gland. To achieve maximal standardization, the component parts of this preparation were examined.

1. *Respiratory activity:* Measurements were begun within ninety to two hundred and forty minutes following exsanguination of the donor animals. In 176 experiments, QO_2 averaged[3] 0.40 ± 0.16. Oxygen consumption slowly declined with prolonged incubation. In 11 experiments, respiratory activity after three hours averaged 84.1 per cent (range: 70.3 to 96.9 per cent) of the initial QO_2. Irreducible delay for processing, transportation and technical preparation precluded the evaluation of an earlier, more precipitous decline of QO_2, such as has been described for myocardial tissue *in vitro* (23).

Prior to incubation, the water content of slices from 54 thyroid glands

[2] During the winters, hyperactive, goitrous thyroid glands were frequently encountered in lambs which had been shorn of wool prior to shipment to the slaughter-house.

[3] Mean $\pm$ Standard Deviation.

averaged 20.3 ± 3.0 per cent.[3] Tissue recovery in 176 experiments averaged 77 ± 14 per cent[3] of the initial weight. Some of the apparent loss may have resulted from an isosmotic redistribution of saline (sodium chloride)[4]. However, turbidity of the suspending medium and overt sediment following centrifugation suggested that weight changes partially represented loss of tissue, presumably due to traumatization at the slice margins. If QO_2 values are corrected for average weight loss and water content, a QO_2 for

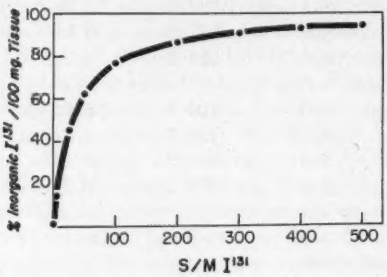

FIG. 1. The disproportionate relationship between S/M I^{131} and accumulation of I^{131} in thyroid slices. Values have been derived on the basis of a medium volume of 3.0 ml. and a constant tissue weight of 100 mg. With increasing values of S/M, progressive depletion of medium I^{131} occurs, so that large changes in the capacity to establish concentration differentials are reflected in only small changes in the percentage of added radioactivity within the tissue.

sheep thyroid slices of 2.6 can be derived, which falls within the range of published values based on final dry weight (24).

2. *Concentration gradients*: Following incubation, differential gradients for I^{131} were established between the slices and the medium. A steady state, with plateau values for S/M I^{131}, was not achieved in the *standard experimental procedure*.[5] However, in 11 experiments in which time relationships could be documented precisely by tipping radioiodine in from the sidearm following temperature equilibration, S/M gradients at the end of one hour

[4] Depression of metabolic activity *in vitro* is associated with an isosmotic increase of cellular hydration (19). Since slices were kept in chilled saline prior to weighing, the initial weights may have been augmented by an imbibition of suspending fluid. Similarly, apparent losses based on wet weight were less when slices were incubated at 38° C. in buffered NaCl, KCl, or in KRP under anaerobic conditions. By drying such slices, it could be shown that preservation of mass was due to increased content of water rather than tissue substance.

[5] *Standard experimental procedure*: Flask contents were removed following eighty to ninety minutes of incubation at 38° C. During this period, twenty to thirty minutes were allotted for gassing and temperature equilibration and sixty minutes for manometric measurements. Variations in S/M ratios due to differences in incubation time were minimized by prompt processing of all flasks at the end of each experiment.

were at least 70 per cent (range: 70.0–100.0) of the values observed at the end of three hours. Detailed analysis of the distribution of I^{131} *in vitro* will be presented in another paper.

S/M I^{131} averaged[3] 48.4 ± 46.9 in 176 studies with the *standard experimental procedure.*

In vivo, following the administration of propylthiouracil, the inorganic I^{131} which is concentrated by the intact thyroid retains the physical properties of ionic iodide and may be discharged by thiocyanate (2, 25). Attempts were made to characterize similarly the I^{131} accumulated by thyroid slices in KRP-Tsp.10^{-3}. Saline homogenates of slices were chromatographed in a collidine-ammonia solvent system (26). As others have shown, the concentrated I^{131} migrated as inorganic iodide (27). In 3 experiments, after gradients for I^{131} had been established between slice and medium, sodium thiocyanate was introduced from the sidearm to yield a final concentration of 10^{-3} M SCN. Addition of thiocyanate was followed by prompt release of the concentrated radioactivity (Fig. 2).

3. *Effects of aging*: Six experiments were performed to assess the effects of aging upon oxygen consumption and iodide transport. Slices were sectioned from fresh thyroid glands and incubated for one hour at 38° C. to determine QO_2 and S/M I^{131}. The remaining intact tissues were stored at 4° C. Following twenty-four to forty-eight hours of such storage, additional slices were prepared. QO_2 and S/M I^{131} were measured and compared to the control values. Representative results are presented in Table 2. Tissues with the lowest initial respiratory activity manifested the least fractional depression after prolonged storage. In general, aging produced a more profound depression of iodide accumulation than of oxygen consumption. The enormous random differences among donor animals can be seen in experiments 167 A, B and C. The three thyroid glands for this study were secured at the same time, from the same lot of animals, and subjected to identical preparative procedures.

TABLE 2. EFFECT OF AGING UPON RESPIRATION AND IODIDE CONCENTRATION BY SHEEP THYROID SLICES

Exp. No.	Control values		Stored samples (% control)	
	QO_2	S/M I^{131}	QO_2	S/M I^{131}
23*	0.22	17.0	73.2	45.1
167 A†	0.39	7.0	74.4	70.0
167 B†	0.67	55.8	65.7	30.3
167 C†	0.83	143.5	61.4	32.8

* Storage at 4° C. for forty-eight hours.
† Storage at 4° C. for twenty-four hours.

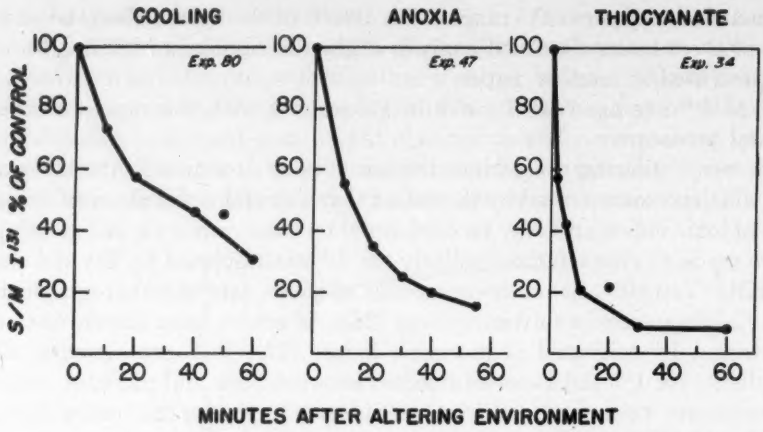

FIG. 2. Discharge of concentrated inorganic I^{131} from thyroid slices by cooling, anoxia, and thiocyanate. Slices were allowed to equilibrate with I^{131} for one hour at 38° C. in an atmosphere of pure oxygen before the environment was altered. In Experiment 34, thiocyanate was introduced from the side-arm to yield a final concentration of 1×10^{-3} M in the main compartment of the Warburg flasks. Temperature of incubation was reduced to 16° C. in Experiment 80. Anoxic conditions were produced in Experiment 47 by flushing the gas phase with 100 per cent nitrogen for five minutes.

4. *Effects of radioactivity*: Addition of 3–4 μc. of I^{131} did not modify respiratory activity in 16 flasks of 4 experiments. Slices were incubated for one hour in 2.7 ml. of nonradioactive KRP-Tap.10^{-3}, and oxygen consumption was estimated. At the end of this time, I^{131} was tipped into the main compartment. Control experiments were performed by introducing similar amounts of KRP-Tap.10^{-3} containing no added I^{131}.

5. *Pooling of lobes:* In 7 experiments, QO_2 and S/M I^{131} of separate lobes from grossly symmetrical glands did not differ significantly (Table 3).

6. *Inhibition of organic binding:* Addition of propylthiouracil or 1-methyl-2-mercaptoimidazole to the suspending medium in concentrations ranging from 10^{-2} to 10^{-5} M did not affect oxygen consumption of the slices. Comparable S/M ratios were achieved with both compounds between 10^{-2} and 10^{-4} M; gradients were varyingly depressed at 10^{-5} M when significant amounts of radioactivity appeared in the organic moieties. At 10^{-5} M, 1-methyl-2-mercaptoimidazole yielded fivefold greater inhibition of organic iodination than did propylthiouracil (Fig. 3). In these studies, 1-methyl-2-mercaptoimidazole was employed because of its greater solubility. Radioactivity in the organic fraction at 10^{-3} M concentrations of blocking agent did not exceed 0.1 per cent of administered counts per 100 mg. of tissue. Since this amount varied with the S/M ratio and was not increased by pro-

TABLE 3. COMPARABILITY OF RESPIRATION AND IODIDE CONCENTRATION BY OPPOSITE LOBES OF SYMMETRICAL SHEEP THYROID GLANDS

Exp. No.	Lobe I		Lobe II/Lobe I	
	QO_2	S/M I^{131}	QO_2	S/M I^{131}
57	0.37	28.2	0.94	1.03
71	0.33	50.2	1.00	1.27
97	0.29	17.5	0.97	1.05
159	0.48	109.5	1.06	1.02
166	0.29	16.9	0.97	1.01
172	0.38	42.2	0.97	1.03
180	0.83	179.7	0.98	0.99

longed incubation over a five-hour period, it was felt that the "organic counts" most probably represented a fractionation artifact rather than true organic binding.

7. *Medium composition:* S/M I^{131} was significantly depressed in a medium composed entirely of NaCl plus phosphate buffer (9.9 mM./L.; pH

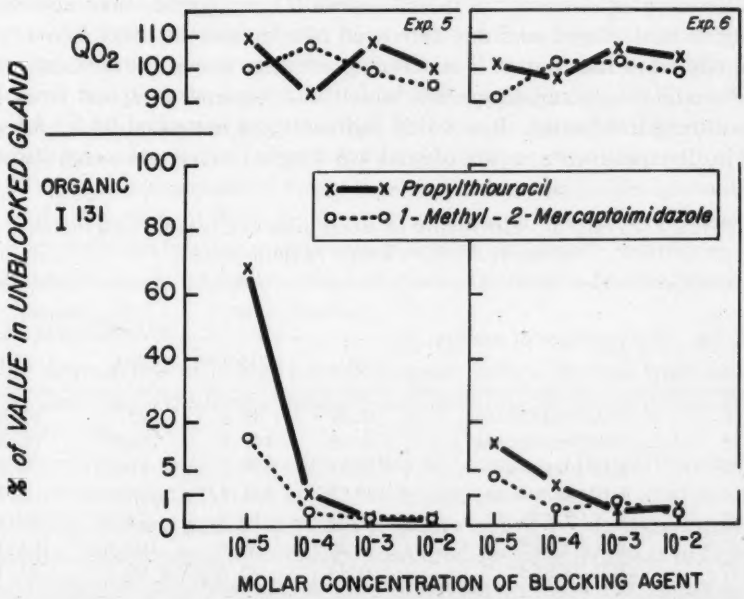

FIG. 3. The influence of antithyroid drugs on the respiration and organic binding of I^{131} by sheep thyroid slices. At 10^{-3} M concentrations of antithyroid agents, less than 0.1 per cent of administered radioactivity was incorporated into the organic fraction.

7.4). In an isotonic KCl-phosphate buffer system, gradients for iodide were virtually abolished without commensurate respiratory depression. "Phosphate-free" media were prepared by substituting tris (hydroxymethyl) aminomethane ("TRIS") for phosphate buffer. Ten experiments were performed with KR-"TRIS"-Tap.10^{-3} at pH 7.4. In 4 studies, QO_2 and S/M I^{131} were unaltered; in 2, both QO_2 and S/M I^{131} were depressed; and in 4 experiments, reduction of S/M I^{131} was unaccompanied by respiratory inhibition. Table 4 demonstrates these relationships.

Although no stable iodide was incorporated into the suspending medium, reagent contamination has been estimated to be 0.1 μg. of I^{127} per ml. (22). Six experiments were performed in which measured quantities of carrier iodide were added to the KRP-Tap.10^{-3}. Progressive diminution of S/M I^{131} ratios occurred at concentrations of I^{127} which exceeded 150–250 μg. per 100 ml.; however, below this level, gradients for I^{131} were unaffected by changes in the medium content of I^{127}. The absolute transport of stable iodide may be computed by multiplying the percentage of radioactivity accumulated within the slices by the total I^{127} initially present within the medium. In the experiment depicted in Table 5, S/M I^{131} remained constant despite a two-hundred fold increase in transported I^{127}.

8. *Recovery:* The counts obtained within the recovered slices and in an aliquot of centrifuged medium were used to estimate the total recovery of administered radioactivity. It was thus possible to assess the accumulation of I^{131} within the small fragments which had become detached from the slices during incubation. Recovered radioactivity averaged 93.5 ± 5.4 per cent[3] in 19 experiments; ratios of final wet weight/initial wet weight for the

TABLE 4. EFFECT OF COMPOSITION OF MEDIUM ON RESPIRATION AND IODIDE CONCENTRATION BY SHEEP THYROID SLICES

Exp. No.	Composition of medium	Control value*		% of control	
		QO_2	S/M I^{131}	QO_2	S/M I^{131}
69	NaCl-phosphate	0.37	30.3	122.7	55.8
211	NaCl-phosphate	0.69	141.4	95.7	38.3
69	KCl-phosphate	0.37	30.3	61.6	4.6
211	KCl-phosphate	0.69	141.4	63.8	2.7
109	KR-"TRIS"†	0.34	19.2	73.5	69.8
136	KR-"TRIS"	0.36	58.6	105.6	104.3
147	KR-"TRIS"	0.44	42.3	99.3	81.1

* Slices from the same gland suspended in Krebs-Ringer-Phosphate.

† Inorganic phosphorus omitted from medium. Tris (hydroxymethyl) aminomethane, pH 7.4, used as buffer.

TABLE 5. THE INFLUENCE OF VARYING CONCENTRATIONS OF INORGANIC IODIDE ON RESPIRATION AND IODIDE TRANSPORT BY SHEEP THYROID SLICES

Flask No.	Initial wt.* tissue (mg.)	Ratio final to initial wt.* tissue	Total recovery of added I^{131} (%)	S/M I^{131}	Flask content of I^{127} (mg. $\times 10^{-3}$)	Added I^{127}† / 100 mg. (%)	I^{127}† / 100 mg. tissue (mg. $\times 10^{-3}$)	QO_2
1	160.5	0.682	74.8	67.3	0.3‡	48.6	0.15	0.33
2	229.0	0.672	71.9	69.9	3.8	39.9	1.52	0.30
3	186.0	0.779	90.3	71.0	7.6	47.5	3.61	0.32
4	173.5	0.683	80.7	37.3	19.0	40.6	7.73	0.31
5	154.5	0.702	84.0	23.8	38.1	35.8	13.63	0.34
6	165.0	0.615	103.7	3.9	190.5	11.9	22.67	0.36
7	201.5	0.717	97.6	2.8	381.0	8.5	32.54	0.33
8	161.5	0.743	103.8	1.0	1905.0	3.4	64.20	0.35
9	189.0	0.648	104.3	0.8	7620.0	2.6	194.31	0.30

* Based on wet weight.

† Present as inorganic iodide.

‡ Estimated contamination of KRP by iodide. Correction for this contamination not included in subsequent calculations.

slices of this series averaged[3] 0.85 ± 0.11. Lack of agreement between the two values may result from: a) false initial weights due to increased cellular hydration (see foregoing sections), b) lower S/M gradients in the detached fragments, or c) discharge of radioactivity into the medium by the cellular debris following removal of the parent tissue. Immediate centrifugation and separation of the medium was instituted to minimize possibility (c). Evidence for definite accumulation of I^{131} by fragments broken off the slices during incubation may be seen in Table 5. At high S/M ratios, recovered radioactivity in flasks 1–5 averaged 80 per cent (*i.e.*, exclusive of the I^{131} within the detached fragments), whereas weight loss averaged 30 per cent. However, in flasks 6–10, although weight loss did not change, recovered radioactivity averaged 103 per cent, since the depressed S/M gradients vitiated the contribution of tissue fragments to total recovery.

II. *Physical factors*

The dependence of iodide transport upon energy derived from cellular metabolism was examined by simple manipulation of the physical factors of the system.

1. *Effect of pH:* PH was varied widely by adjusting the phosphate buffer in the suspending KRP-Tap.10^{-3}. Supplemental sodium bicarbonate (5 to 20 mEq./L.) was added to yield pH values above 7.8. In 3 experiments, slices also were incubated in "TRIS"-buffered systems to assess pH effects in the absence of phosphate or added bicarbonate anions. PH values of the suspending media were measured with a Beckman glass electrode before and after incubation. In acid solutions, parallel increases of S/M I^{131} and QO_2 were observed as final pH approached 7.0 to 7.4. Above pH 7.4,

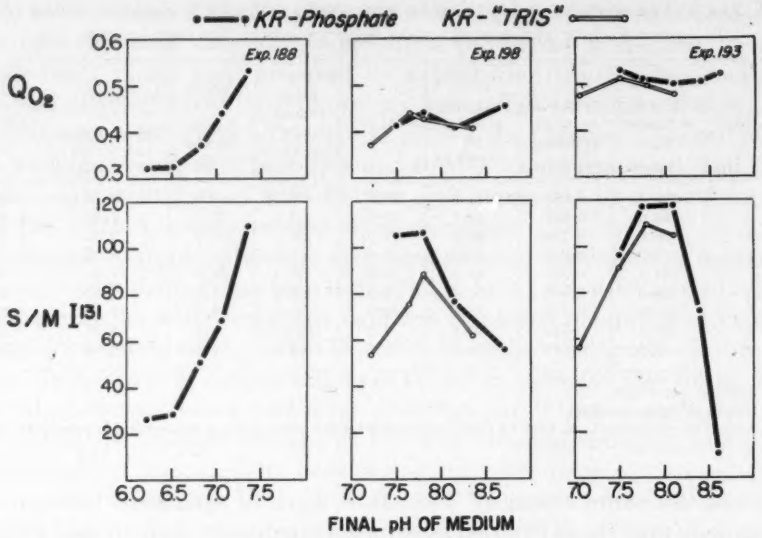

FIG. 4. The effect of pH on the respiration and concentration of iodide by sheep thyroid slices. Supplemental $NaHCO_3$ (5–20 mEq./L) was added to phosphate buffer to yield pH values above 7.8. Tris (hydroxymethyl) aminomethane was used as buffer in the preparation of "phosphate-free media" (KR-"TRIS"). All media contained 10^{-3} M concentrations of Tapazole. pH values of the suspending solutions before and after incubation were: Exp. 188: 6.04, 6.22; 6.47, 6.52; 6.98, 6.82; 7.27, 7.11; 7.68, 7.35. Exp. 198 KRP: 7.64, 7.49; 7.95, 7.79; 8.32, 8.15; 8.80, 8.71. Exp. 198 KR-"TRIS": 7.30, 7.21; 7.76, 7.64; 8.18, 7.80; 8.83, 8.35. Exp. 193 KRP: 7.55, 7.49; 7.87, 7.74; 8.18, 8.08; 8.72, 8.61. Exp. 193 KR-"TRIS": 6.99, 70.4; 7.70, 7.49; 7.91, 7.78; 8.29, 8.10.

gradients for I^{131} continued to rise without significant augmentation of oxygen consumption. Both in KRP and in KR-"TRIS", maximal S/M I^{131} ratios were observed at a final pH variously ranging from 7.6 to 8.2. Iodide concentration was depressed in 4 experiments in which the final pH exceeded 8.6 (Fig. 4).

2. *Effect of temperature:* Virtually no oxygen consumption or accumulation of iodide could be demonstrated at 5° C. Iodide transport and respiratory activity increased in parallel as the temperature of incubation was raised to 38° C. Both S/M I^{131} and QO_2 were approximately doubled with every rise of 10° C. in temperature. At higher temperatures, disruption of cellular integrity or relative anoxia may be anticipated. Thus, above 38° C., a limited range was observed wherein QO_2 could be further augmented without commensurate elevation of concentration differentials. Both processes decreased above 45° C. (Fig. 5). Incubation temperature affected not only the absolute magnitude of S/M but also the speed of equilibration.

TABLE 6. REVERSIBILITY OF THE EFFECTS OF COOLING AND ANOXIA UPON THE ACCUMULATION OF IODIDE BY THYROID SLICES

Time (min.)	Experimental environment	Flask No.				
		1*	2*	3*	4†	5†
0–60	38° C.; 100% O_2	+‡	+	+	+	+
60–90	38° C.; 100% N_2		+		+	
	4° C.; 21% O_2			+		+
90–150	38° C.; 100% O_2				+	+
Per cent of control S/M I^{131} (183.1)		100	10.5	34.3	105.0	94.5

* I^{131} tipped in from side-arm at 0 minutes.

† I^{131} tipped in from side-arm at ninety minutes.

‡ Indicates the experimental environments in which the flasks were successively incubated. Slices were removed from flask 1 at the end of sixty minutes, from flasks 2 and 3 at the end of ninety minutes, and from flasks 4 and 5 at the end of one hundred and fifty minutes.

The role of metabolic activity in maintaining established concentration differentials was evaluated by immersing flasks in cracked ice after equilibration at 38° C. Prompt discharge of accumulated iodide occurred (Fig. 2). Gradients were quantitatively re-established when the temperature was restored to 38° C., thus demonstrating the complete reversibility of the "cooling" phenomenon[6] (Table 6).

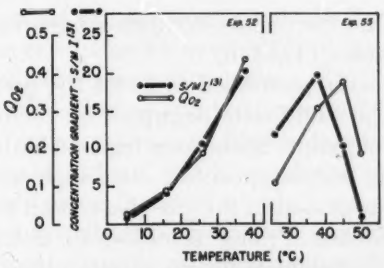

FIG. 5. The effect of temperature on the respiration and concentration of iodide by sheep thyroid slices.

[6] It should be noted that these experiments duplicated a sequence of events intrinsic to the *standard experimental procedure*. Since flasks were routinely chilled on cracked ice during additions of tissue, the slices presumably discharged their stable inorganic iodide into the suspending medium during the preparative manipulations. Thereafter, the establishment of S/M gradients for I^{131} at 38° C. also must have reflected a reaccumulation of I^{127} by the slices.

3. *Effect of oxygen:* In a system dependent upon diffusion for distribution, maximal S/M ratios of about 0.3 might be anticipated if iodide were confined to the extracellular phase; and of 0.8, if iodide exchanged with total water. S/M^{131} ratios of 1.1–1.9 were observed in 8 experiments in which slices were incubated in 100 per cent nitrogen for one hour. Slices from the same glands concentrated ten to fifty fold in 100 per cent oxygen. In 3 of 5 experiments, S/M ratios were 10 to 25 per cent higher in pure oxygen than in room air; substitution of oxygen for ambient air in the gas phase uniformly enhanced QO_2 (Fig. 6). The participation of oxidative processes in the retention of iodide was assessed by flushing nitrogen into the gas phase following aerobic establishment of S/M gradients. Concentrated radioactivity was rapidly discharged (Fig. 2). Complete reaccumula-

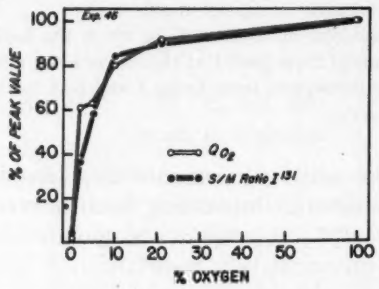

FIG. 6. The effect of varying oxygen tensions on the concentration of inorganic I^{131} by thyroid slices.

tion of I^{131} occurred in experiments in which oxygen was reintroduced after thirty minutes of anoxia (Table 6).

Two experiments were performed to assess the possibility that cellular organization might be sufficiently disrupted by prolonged anoxia to preclude the retention of iodide. Slices were exposed to 100 per cent nitrogen at 38° C. for varying intervals; control slices were incubated in room air. Oxygen was then substituted in the gas phase of all flasks and I^{131} was introduced from the sidearm. Prior anaerobiosis for as long as forty-five minutes did not affect subsequent oxygen consumption or iodide transport. Even following exposure to nitrogen for one hour, QO_2 was at most 20 per cent less than in control flasks, and S/M ratios were depressed only 30 to 40 per cent.

DISCUSSION

Studies have been performed with a preparation which concentrates I^{131} in a form that is trichloracetic-acid soluble and chromatographically indistinguishable from inorganic iodide. Extension of results obtained *in vitro*

to complex phenomena *in vivo* is always hazardous. Nonetheless, iodide transport in the thyroid slice simulates many of the characteristics of iodide transport in the intact animal. First, following variable periods of equilibration, rising gradients are established between tissue and extracellular fluid (28). Secondly, S/M ratios are not modified by minor changes in the volume of medium or in its content of stable or radioactive iodide (2, 11). Finally, prompt discharge of concentrated radioactivity may be effected by the introduction of thiocyanate (2, 25).

The use of thyroid slices facilitates manipulations which cannot be performed *in vivo*. For example, acute depression of oxidative activity by cooling or anoxia reversibly interrupts established concentration differentials. Since retention of inorganic iodide cannot be dissociated from accumulation, the term "iodide trap" with its connotation of unidirectional flux and sequestration does not validly characterize the underlying phenomena. Neither does simple exchange diffusion, since net accumulation of iodide can be demonstrated by re-establishing physical optima of temperature and gas tension or by varying the content of carrier I^{127} within the medium.

In the slice preparation, continued aerobic cellular activity is prerequisite for the establishment and the maintenance of S/M gradients. The data do not permit differentiation between the metabolic dependencies for transcellular penetration, intracellular concentration, or intrafollicular localization of ionic iodide. However, the integrated processes must be viewed as an example of active transport. Such an interpretation justifies re-examination of certain published observations. Thus, progressive anoxia, cooling, and pH changes could explain the declining thyroid/plasma ratios for I^{131} which have been reported in mice from which the thyroid glands were excised at varying intervals following death (28). Similarly, it is not surprising that during twenty-four hours of dialysis at 5° C., thyroid homogenates failed to accumulate I^{131} which could be discharged by thiocyanate (29).

The designation "active transport" implies specificity. In the present experiments, iodide transport and cellular aerobic metabolism (as measured by QO_2) are intimately associated over a wide range of experimental variables. The greatest differentials between slice and medium generally are established by the most actively respiring tissues. However, among different animals, S/M I^{131} varies more than QO_2, and considerable evidence would indicate that oxidative processes supply only the minimal requirements of energy for transport. Thus QO_2 and S/M I^{131} may be dissociated to demonstrate other, possibly more specific, limiting factors. The disparate deterioration of transport following storage at 4° C. may be cited as an example. Similarly, iodide accumulation is moderately depressed in buffered sodium chloride and virtually abolished in buffered potassium chloride, although respiration is unaltered or only partly reduced. The lat-

ter experiments suggest, but do not prove, that intracellular cations (18, 30) may modify the transport performance of thyroidal slices. Environmental pH can also affect S/M I^{131} independent of oxidative mechanisms. Between pH 7.2–8.6, respiratory activity does not change appreciably; however, gradients for iodide fluctuate markedly. At pH slightly greater than 7.4, maximal transport is observed. Whether this results from transformation of ion characteristics and stable cellular components, or from biochemical alterations in selective, unstable systems cannot be answered at present.

Iodide transport is of special importance in thyroidal economy. Ionic iodide is the substrate for the oxidative phenomena of organic iodination. Thus, the ability to concentrate iodide may act as a rate-limiting factor in the synthesis of hormone, since the availability of substrate will be determined by the magnitude of concentration differentials for iodide between thyroid and perfusate. Others have incubated unblocked thyroid slices under many conditions of metabolic inhibition (31, 32). The resultant impaired synthesis of thyroxine may be explained, at least partially, by a limitation of iodide. It is apparent that organic iodinations can be studied in cellular systems only if experimental variables do not alter the accumulation of ionic substrate.

SUMMARY

Metabolic dependencies of iodide transport have been studied *in vitro*. Concentration of inorganic I^{131} by surviving slices of sheep thyroid tissue was examined in the Warburg apparatus and related to simultaneous estimation of cellular oxygen consumption. Organic binding was inhibited by the inclusion of 1-methyl-2-mercaptoimidazole in the suspending medium. In this preparation, cellular radioactivity retained the characteristics of ionic iodide with respect to solubility, chromatographic mobility, and discharge by thiocyanate.

It has been shown that minimal iodide transport requires the continuous expenditure of energy derived from aerobic cellular metabolism. Limiting factors, in addition to oxidative processes, were demonstrated by aging of tissues or by modification of environmental pH or electrolyte composition. These findings have implicated an active, metabolically-linked process as a participating component in the thyroidal transport of inorganic iodide against a concentration gradient.

Acknowledgments

The technical assistance of Miss Betty Lou Spear is gratefully acknowledged. We are indebted to Dr. John V. Taggart for his advice and interest in this work. Tapazole was generously supplied by Dr. C. D. Hines of the Lilly Research Laboratories and Dr. E. B. Astwood; propylthiouracil by Dr. James M. Ruegsegger of the Lederle Laboratories. Sheep and lamb thyroid glands were obtained through the kind co-operation of Mr. Harold N. Larson of the New England Dressed Meat and Wool Co., Somerville, Mass.

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HISTOPATHOLOGIC EXAMINATION OF THE SKIN IN THE DIAGNOSIS OF MYXEDEMA IN CHILDREN

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FOR years it has been known that hypothyroidism may be associated with an accumulation of a mucinous substance in the dermal connective tissue. In 1878 Ord (1) coined the term myxedema, *i.e.*, mucinous edema, which has ever since been used to designate the entire syndrome caused by hypofunction of the thyroid gland. In a case of hypothyroidism Ord, by *chemical* methods, found fifty times the normal amount of mucin in the skin. A few years later (1885) Horsley (2) reported accumulation of mucin in the connective tissue of myxedematous humans and monkeys.

Carol (3) studied the skin of patients with localized myxedema by *chemical* methods and found a mucin-like substance which reduced Fehling's solution following hydrolysis with hydrochloric acid. Watson (4) and Watson and Pearce (5) reported a marked increase in the connective-tissue content of hyaluronic acid and chondroitin sulphuric acid in localized myxedema. In the tissues, these mucopolysaccharides are bound to proteins. Watson and Pearce (6) found the hyaluronic acid content to be thrice, and the chondroitin sulphuric acid content to be twice that in normal skin.

Pretibial localized myxedema appears to be in every respect of the same nature as the changes encountered in hypothyroidism, only more pronounced (Reuter (7), Asboe-Hansen (8)).

By the *histochemical* approach numerous workers (among others, Unna (9), Kreibich (10) and Reuter (7)) have demonstrated an accumulation of mucinous substances in myxedematous skin.

Asboe-Hansen (8, 11), using histochemical methods, confirmed Watson and Pearce's finding of increased hyaluronic acid and chondroitin sulphuric acid. No one has yet succeeded in proving the presence of altered quantities of hyaluronidase in the connective tissue in cases of myxedema. Trotter and Eden (12) suggested that a reduced hyaluronidase activity might account for the condition, but they did not advance any proof of this contention.

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Since hyaluronic acid has a marked capacity to bind water (Ropes *et al.* (13)), its accumulation in the tissues explains the large water content. These alterations in the connective tissue are not signs of degeneration, but of lively cellular activity, and the condition is reversible.

Myxedematous connective tissue shows accumulations of mast cells, partly in a perivascular position and partly free in the tissue. Several investigations indicate that these cells are the origin of hyaluronic acid in the mesenchymal tissues. Chemical and histochemical studies have shown that they contain a mucopolysaccharide and that they are the only type of cell in the connective tissue containing this substance. This mucopolysaccharide is chemically closely related to heparin and hyaluronic acid, presumably their precursor (Asboe-Hansen (8–11)).

Pathologic accumulations of mucinous substances in the connective tissue occur when the function of the pituitary is increased in relation to the thyroid gland. This state is in most cases a result of reduced inhibition, due to a reduced production of thyroxine from the thyroid gland. The thyrotropic hormone is known to act directly on the connective tissue, without the intermediary of the thyroid gland. This view is supported by a number of biologic animal experiments (Ludwig, Boas and Soffer (14), Asboe-Hansen and Iversen (15)) and various studies on the thyrotropin level in the blood and urine of patients suffering from thyroid disorders. For example, some myxedematous subjects have exhibited an increased urinary excretion of thyrotropin. On the other hand, urinary thyrotropin has been found to be reduced or inactivated in thyrotoxicosis (van Caulaert *et al.* (16), Hertz and Oastler (17), Rawson *et al.* (18)). D'Angelo *et al.* (19) and Wichmann (20) also found increased thyrotropin levels in the blood of myxedematous patients.

On the basis of these investigations, the therapeutic effect of the thyroid hormone in myxedema is perhaps interpretable as an inhibition of the thyrotropic activity of the pituitary.

In addition to the accumulation of mucinous substances in the connective-tissue ground substance and the accumulation of mast cells which contain kindred mucinous substances, several other signs are more or less constant. In extreme degrees of myxedema, the collagen bundles of fibrils and the elastic fibers are, as it were, burst apart, appearing in a very irregular and loose arrangement (Reuter (7), Asboe-Hansen (8)). The collagen fibrils may be degenerated, swollen and basophilic. The degree of edema varies; in severe cases the edematous changes are striking. The epidermis shows degenerative changes and nearly always some degree of keratosis.

Studying patients with a low metabolic rate but without visible myxedema, Reuter (7) found changes similar to those present in the more se-

vere cases, but no clear signs of edema. Reuter did not believe that the histopathologic appearance was diagnostic, because "mucin probably is a normal constituent of the skin."

Normal dermal connective tissue may stain metachromatically with toluidine blue. This, however, only applies to the papillary layer, whereas the deeper parts practically always stain faintly. Many normal subjects exhibit practically no metachromasia of the connective tissue, not even children, who generally possess more metachromatic substance than adults. Increased quantities of metachromatic substance may be found in certain morbid conditions of the skin, such as keloids, some itching skin diseases, lupus erythematosus, exfoliative erythroderma and several other disorders.

TECHNIQUE

Biopsy specimens of the skin were obtained in all cases of the present series from the dorsal aspect of the upper arm, three finger-breadths above the olecranon process. None of the patients suffered from skin disorders which might have disturbed the evaluation of the histologic preparation. The biopsy specimens were removed by a punch (3 mm. in diameter), fixed in a 4 per cent solution of basic lead acetate by the method of Holmgren, and stained in a ½ per cent aqueous solution of toluidine blue. By means of a buffer solution, the pH of the fluid was kept constant at 4.5–5. The staining period was thirty minutes.

A priori, it was impossible to tell whether the accumulation of mucin in the tissues was applicable as a diagnostic sign of myxedema in children. Metachromatic staining with toluidine blue is a fairly constant and fairly specific reaction on the part of mucopolysaccharide—hyaluronic acid and chondroitin sulphuric acid, in the skin. The part which is eliminated by hyaluronidase is probably due to hyaluronic acid, as, according to Meyer and Rapport (21), the chondroitin sulphuric acid of the dermal connective tissue is not susceptible to hyaluronidase.

By histologic methods it is difficult to evaluate this substance quantitatively, but if such factors as the thickness of the sections, and the pH of the fluids used during preparation and staining are kept constant, it is possible—with considerable experience—to classify the metachromasia into degrees: 1) highly pathologic, 2) slightly pathologic, and 3) not pathologic. In addition, the number and granulation of the mast cells, the edema, the state of the blood vessels, the texture of the fibrils, and the degree of keratosis should be taken into consideration.

The designation "+" indicates preparations in which the metachromasia, and the number of mast cells were highly increased as compared with normal; "(+)" indicates morbid, but less marked changes; and "−" indicates normal skin.

The present study was designed to estimate the value of the foregoing criteria in diagnosing myxedema in childhood. It could not be taken for granted that the skin in congenital thyroid aplasia would exhibit the changes similar to those observed in the acquired myxedema of adults. In children, even more than in adults, it is important to have simple and specific means of early diagnosis and of controlling the adequacy of the treatment. In our opinion, there is still a need for such means, even after the introduction of methods for determining thyroidal uptake of radioactive iodine and serum protein-bound iodine concentration.

All the histologic examinations were carried out by Asboe-Hansen, whereas the clinical diagnoses were made by Andersen and Quaade. The histologic and clinical diagnoses were made independently of each other, and the results were not compared until the study was concluded.

CLINICAL MATERIAL

The series comprised 99 children, ranging in age from 3 months to 15 years, from various children's hospitals and out-patient departments in Copenhagen. A distinction was made between the following three groups:

1. *Definite clinical signs of hypothyroidism (26 children).* As we felt that it was essential to define a group of children in whom the diagnosis of hypothyroidism was beyond doubt, this group includes only patients who fulfilled the ordinary criteria of thyroid hypofunction and who exhibited nothing of any relevance which could contradict the diagnosis. Particular stress was laid on the following signs: stunted growth, retarded osseous development and dentition, epiphyseal dysgenesis, the appearance of the skin, the temperature and moisture of the skin, typical facies, bodily proportions and appearance on the whole, bradycardia, low pulse pressure, constipation, subnormal temperature, increased sensitivity to cold, sluggish mentality and low intelligence. All the children were observed in the hospital for long periods and all gave a convincing response to the administration and withdrawal of thyroidin.

2. *Suspected hypothyroidism (21 children).* In these children a definite diagnosis could not be made on the basis of the foregoing criteria. The clinical data in this group are given in Table 1.

3. *No signs of thyroid hypofunction (52 children).*

Table 2 shows the results of skin biopsy in the 3 groups of children. In the cases in which the findings were + or (+), the changes yielded to administration of thyroidin given for a minimum of one month. Furthermore, the typical histologic appearance returned after thyroidin had been withheld for the same period.

The relationship between the thyroidin therapy, the time of the biopsy, and the result of the histologic examination in Group 1 is given in Figure 1.

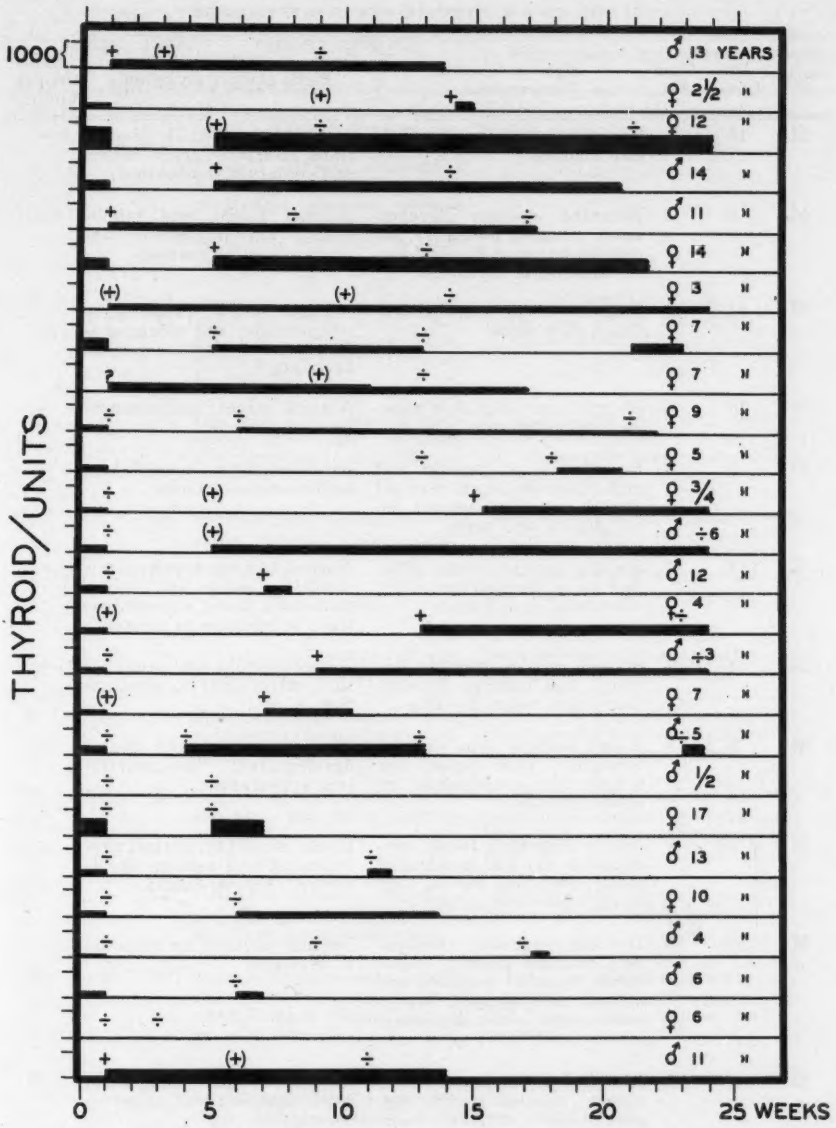

FIG. 1. Relation between skin biopsy findings and thyroidin therapy in a group of children showing definite clinical signs of hypothyroidism.

TABLE 1. COMPARISON OF SKIN BIOPSY FINDINGS AND CLINICAL DATA IN 21 CHILDREN WITH SUSPECTED HYPOTHYROIDISM

Sex	Age (yrs.)	Case No.	Signs in favor of myxedema	Signs against myxedema	Skin biopsy findings
M	13	1	Constipation, slow pulse, sluggish mentality	Normal height, bodily proportions, skin, intelligence, facies, and centers of ossification	−
M	7	2	Retarded osseous development, sluggish mentality, increased serum cholesterol level; response to thyroidin	Normal height and intelligence; uncharacteristic appearance; pulse normal	(+)
M	14	3	Stunted growth, retarded dentition, slow pulse	Normal bodily proportions, temperature, and serum cholesterol level; uncharacteristic appearance	−
M	10	4	Sluggish mentality, slow pulse, cool and rough skin	Normal height; uncharacteristic appearance	−
M	9	5	Retardation of psychic and motor development, stunted growth, retarded osseous development, slow pulse	No constipation; normal skin; uncharacteristic facies	−
M	13	6	Stunted growth, rough skin, slow pulse, sluggish mentality, subnormal temperature	Normal osseous development, dentition, pulse and serum cholesterol level; no constipation; no response to thyroidin	−
M	11	7	Stunted growth, retarded dentition and osseous development, slow pulse, dry skin	Normal psyche and temperature; warm skin; no constipation	+
M	6	8	Rough and dry skin, sluggish mentality, slow pulse, low temperature, constipation, arrested growth	Normal osseous and dental development; uncharacteristic appearance	−
M	4/12	9	Highly suggestive facies, constipation, dry and rough skin, pallor, umbilical hernia, high serum cholesterol level	Lively mentality, normal psychomotor and osseous development, normal height	−
M	10	10	Dry and rough skin, constipation, stunted growth, coarse facies, retarded dentition and osseous development, bradycardia, high serum cholesterol level	Normal psyche; no response to thyroidin	−
M	2	11	Retarded psychomotor development, stunted growth, suggestive facies, dry skin, rough and hoarse voice, retarded development of ossification centers; response to thyroidin	Normal serum cholesterol level, dentition and pulse; no constipation	−

TABLE 1 (*Continued*)

Sex	Age (yrs.)	Case No.	Signs in favor of myxedema	Signs against myxedema	Skin biopsy findings
M	7	12	Retarded psychic development, stunted growth, retarded dentition, scaly, rough, dry and cold skin, abnormally sensitivity to cold, sluggish mentality, retarded osseous development, subnormal temperature; response to thyroidin	Facies not typical; normal serum cholesterol level; no constipation	−
F	3	13	Retarded osseous development, alteration of skin, hair and voice, suggestive facies, sluggish mentality; response to thyroidin	Normal height, dentition, pulse and serum cholesterol level; no constipation	(+)
F	8	14	Dry, cool skin, sluggish mentality, slow pulse, constipation; response to thyroidin	Normal height, dentition, osseous development, and serum cholesterol level; uncharacteristic facies	(+)
F	12	15	Stunted growth, sluggish mentality; response to thyroidin	Normal osseous development, serum cholesterol level, temperature, pulse rate, bodily proportions, and skin; normal puberty; facies uncharacteristic	(+)
F	3/12	16	Constipation, retarded growth, subnormal temperature	Normal osseous development; uncharacteristic appearance	(+)
F	13	17	Slow pulse, dry and cool skin, subnormal temperature; response to thyroidin	Normal height and osseous development; no constipation; uncharacteristic appearance	−
F	12	18	Stunted growth, constipation, cool skin, slow pulse; response to thyroidin	Lively mentality; normal osseous development, temperature, and serum cholesterol level	+
F	2	19	Retarded osseous development, suggestive facies, constipation, sluggish mentality	No response to thyroidin; normal serum cholesterol level	−
M	2	20	Suggestive appearance, hoarse voice, retarded motor development, sluggish mentality, cool and dry skin	Normal serum cholesterol level, pulse, osseous development, and height; no response to thyroidin	−
F	8	21	Increased serum cholesterol level, suggestive facies, slow pulse, fatigue, sluggish mentality, yellowish and rough skin	Normal height; no constipation	+

TABLE 2. RESULTS OF SKIN BIOPSIES IN THE THREE GROUPS OF CHILDREN

Group	No. of children	Positive biopsy findings + or (+)	Negative biopsy findings
#1. Definite myxedema	26	15	11
#2. Doubtful myxedema	21	8	13
#3. No myxedema	52	0	52

In Table 1 the results of the skin biopsies are compared with the clinical data in the children with doubtful hypothyroidism (Group 2).

As regards Group 3, comprising 52 euthyroid children, the results of the skin biopsies were negative in all cases. The diagnoses in this group were as follows: normal, 4; obesity, 14; mental deficiency, 11; dwarfism, 6; mongolism, 5; juvenile goiter, 2; Graves' disease, 1; dyschondroplasia, 1; rheumatoid arthritis, 1; Turner's syndrome, 1; muscular dystrophy, 1; dermatomyositis, 1; myositis, 1; muscular infiltration, 1; edema, 1; and xanthelasmata, 1.

DISCUSSION

It is possible that the following factors may have caused the negative result of the histologic examination in nearly half the children with definite clinical signs of hypothyroidism:

1. Failure to withdraw thyroidin therapy in children treated in outpatient departments. The children whose mothers admitted such failure were excluded.

2. Thyroidin may have been withheld for too brief a period. According to our latest experience, the histologic changes may take up to six weeks to re-appear, especially following overdosage of thyroidin.

3. Provided increased metachromasia and accumulation of mast cells in the skin are results of the action of thyrotropic hormone—as indicated by many findings—a negative result of histologic examination must be expected in cases of pituitary insufficiency. As there was no possibility of examining the patients by determining thyroidal I^{131} uptake, serum protein-bound iodine concentration, or the blood level of thyrotropic hormone, we did not feel justified in distinguishing between pituitary and thyrogenic hypothyroidism.

CONCLUSION

The histologic findings on examination of a skin biopsy specimen were positive in more than half the children with unmistakable clinical hypothyroidism, whereas they were negative in children without a reduction in thyroid function. In a group of 21 children with doubtful hypothyroidism,

the findings were positive in 8. When compared with previous reports on hypothyroidism in adults, our results warrant the following conclusions:

1. A skin biopsy specimen which shows the foregoing characteristic changes appears to afford a reliable diagnostic criterion for hypothyroidism, provided certain local dermal changes can be ruled out.
2. Negative skin biopsy findings do not exclude hypothyroidism.

SUMMARY

The histologic structure of the dermal connective tissue in hypothyroidism is described, with special reference to the ground substance, mast cells, fibrillar structure, vessel walls, and epidermis, as well as the action of the thyrotropic hormone on these tissue elements.

Histologic examination is evaluated as a diagnostic test of hypothyroidism, and the technique is described.

By this method 99 infants and children were examined. The results were as follows:

In 15 of 26 patients with definite clinical hypothyroidism the skin biopsies showed changes in the epithelial and connective tissue structures of the skin. In 8 of 21 patients suspected of hypothyroidism skin biopsies showed similar findings. A control group of 52 euthyroid children had normal skin.

In all patients with a pathologic condition of the skin, the changes disappeared during administration of thyroidin, and could be reproduced by its withdrawal.

The possible causes of negative results in hypothyroidism are discussed.

The method is a simple and handy aid in the diagnosis of hypothyroidism, especially in children. Positive findings seem to indicate hypothyroidism; negative findings do not exclude it.

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FAMILIAL ATHYREOTIC CRETINISM: REPORT OF 3 CASES

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THREE types of sporadic cretinism have been described in the literature—non-endemic goitrous, secondary (pituitary), and athyreotic. I. *Non-endemic goitrous hypothyroidism.* Occasionally enlarged thyroid glands have been noted in cretins living in regions where goiter is not endemic. Hypothyroidism in these patients is due to an inability of the gland to produce thyroid hormone, even though it can accumulate iodine readily as shown by studies with I^{131} (1–6). The enlargement of the thyroid is presumed to be due to an increased secretion of thyrotropic hormone secondary to deficiency of circulating thyroid hormone. It is relatively common for this form of cretinism to be familial (1, 7). II. *Secondary (pituitary) hypothyroidism.* Cretinism of the sporadic type also may be secondary to a deficiency of anterior pituitary thyrotropic hormone production. This type is usually, but not necesarily, associated with signs of a generalized pituitary insufficiency. III. *Athyreotic cretinism (athyreosis).* Most instances of sporadic cretinism are of this type, which is due to a defect in the embryonic development of the thyroid gland so that the gland is entirely absent or only rudimentary. This form of sporadic cretinism is not considered to be genetically determined, since it rarely appears in more than one member of the same family; moreover, in heterozygous twins, one child may be normal and the other a cretin (8). The purpose of this paper is to report the unusual occurrence of athyreotic cretinism in 3 siblings.

SUBJECTS AND STUDIES

The subjects of this study were 3 siblings of Mexican-American descent, ages 8 (Z.G.), 6 (T.G.), and 3 (Te.G.) years, born of successive term pregnancies. There was 1 older male sibling, aged 10 years, who was apparently normal. The father and mother were first cousins and the family history was strikingly suggestive of cretinism (Fig. 1).

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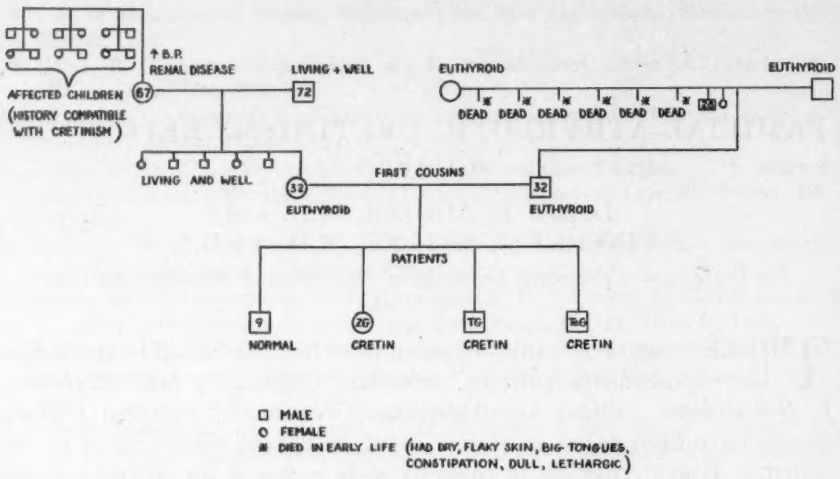

FIG. 1: Family history of Patients Z.G., T.G. and Te.G.

Each of these children had a history of lethargy, constipation and slow physical and mental growth. Each had the typical features of cretinism. Salient findings on physical examination were the persistence of infantile body proportions; coarse, dry hair; dry seborrheic skin; macroglossia; delayed, hypoplastic dentition; protuberant abdomen with persistent umbilical hernia; short, spadelike hands; and myxedematous tissue deposits. None had a palpable thyroid gland. Patient Te.G. also had a cleft of the soft and hard palates. The appearance of these children prior to the institution of therapy is shown in Figure 2A and their appearance after five months of therapy with desiccated thyroid is shown in Figure 2B.

The diagnosis of cretinism was substantiated by the results of serum cholesterol and protein-bound iodine (9) determinations and by the clinical response to thyroid administration. Table 1 shows the serum cholesterol determinations obtained before therapy, during the fifth month of therapy with desiccated thyroid, and after thyroid therapy had been interrupted for

TABLE 1. SERUM CHOLESTEROL LEVELS (MG./100 ML.)

Patient	Before therapy	During therapy*	After therapy†
Z.G.	295	230	364
T.G.	305	240	366
Te.G.	240	210	444

* After desiccated thyroid had been administered for five months.

† Ten months after desiccated thyroid administration had been interrupted.

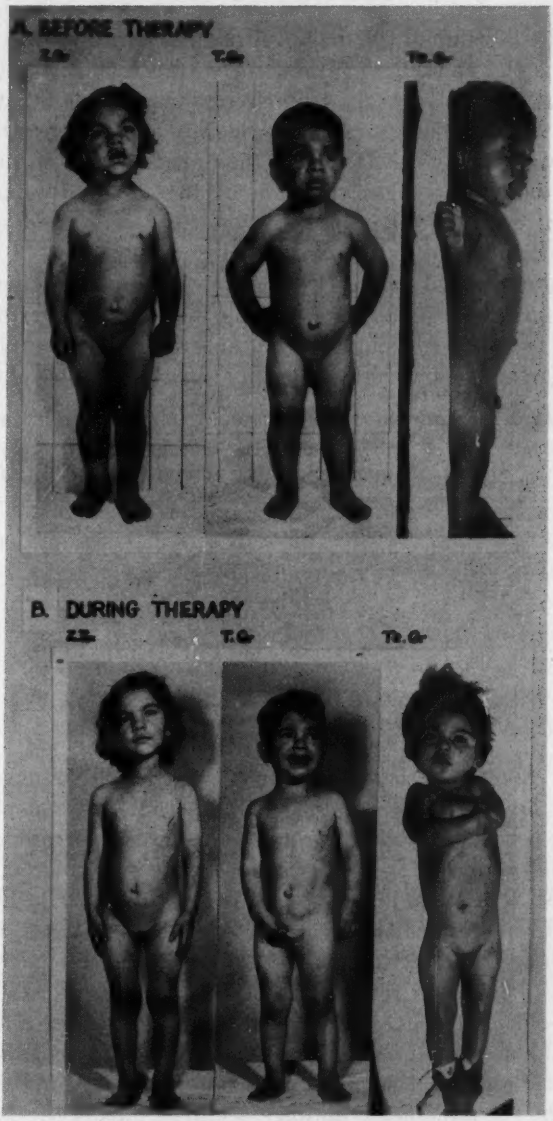

FIG. 2: **A.** Patients Z.G., T.G. and Te.G. before thyroid therapy. **B.** Patients after five months of therapy with desiccated thyroid.

a period of ten months. Although the serum cholesterol level is frequently elevated in untreated hypothyroidism, single or even multiple determinations are of little value in the diagnosis of this condition. Of much greater significance is the marked elevation in the serum cholesterol level which occurs from six to twelve weeks after thyroid medication is withdrawn (10, 11). In each of these patients prior to therapy the concentration of serum cholesterol was slightly elevated or at the upper limits of normal, and during therapy there was a slight, questionably significant decrease. However, ten months after thyroid therapy had been withdrawn, there was a definite elevation in the level of serum cholesterol.

Serum protein-bound iodine (PBI) concentrations were determined before, during and after therapy, at the same intervals as described for serum cholesterol (Table 2). Although the initial serum PBI concentrations were low, they were not so low as those usually reported in athyreotic cretinism.

TABLE 2. SERUM PROTEIN-BOUND IODINE LEVELS (μG./100 ML.)

Patient	Before therapy	During therapy*	After therapy†
Z.G.	2.75	6.3	0.4
T.G.	3.0	4.1 5.1	0.2
Te.G.	1.7	11.6 11.7	1.9

*† See footnotes to Table 1.

That these values may have been erroneously high is suggested by the consistently lower results obtained in numerous subsequent determinations during periods in which thyroid therapy was interrupted.

During the period of therapy with desiccated thyroid (U.S.P), definite improvement in physical appearance was noted (Fig. 2B). There was also an increased rate of linear growth in each of these children, and the eruption of teeth occurred in the youngest child. Prior to therapy, roentgenograms of the bones had revealed markedly delayed skeletal maturation. The 8-year-old child (Z.G.) had a bone age of 3 years and the other 2 children had a skeletal maturation comparable to that of the normal newborn infant. Epiphyseal stippling was not apparent on these films. No change in the roentgenologic findings occurred during the brief period of therapy.

To identify the type of hypothyroidism, the following tests were performed:

1. After thyroid medication had been interrupted for a period of ten

TABLE 3. RESPONSE OF THE SERUM PBI TO THYROTROPIN

Patient	Serum protein-bound iodine (μg./100 ml.)				
	Control	1 day*	3 days*	5 days*	10 days
T.G.	0.2	0.0	0.0	0.0	0.0
Te.G.	1.9	0.0	0.0	0.0	0.0
Z.G.	0.4	0.0	0.75	1.5	0.75

* During the 5-day period of thyrotropin administration.

months, the serum PBI concentration was determined.[1] The values were extremely low, being 0.2, 1.9 and 0.4 μg. per 100 ml. Thyrotropic hormone then was administered intramuscularly in a dose of 4 units every eight hours for five days. Serum samples for PBI determinations were obtained at one, three, five and ten days. No significant changes in the PBI levels occurred during this period (Table 3).

2. At a subsequent time, when thryoid medication had been withheld for a period of three weeks, radioiodine uptake studies were made.[2] Radioiodine was administered orally in a dose of 8 microcuries and triplicate counts were obtained twenty-four hours later at 21-cm. distance for a period of two minutes. Counts were obtained in three areas of the thyroid region: the center of the neck, 1 cm. below the cricoid cartilage, and over the suprasternal notch. Control counts were obtained at two locations on each thigh: 5 cm. below the groin, and 5 cm. above the knee. In each patient a failure to concentrate radioiodine in the thyroid was demonstrated; counts obtained over the thyroid area did not differ from those obtained over the thighs.

DISCUSSION

The diagnosis of cretinism in these 3 siblings was made clinically and was confirmed by laboratory procedures, including determinations of serum PBI concentration and thyroidal I^{131} uptake. During therapy with desiccated thyroid the clinical and laboratory abnormalities which made the diagnosis of cretinism possible became less marked and in most instances disappeared.

Until recently the only means of differentiation between hypothyroidism of thyroid origin and that of pituitary origin has been through detection of signs of other pituitary deficiencies, as manifested by severe growth re-

[1] Determinations were performed by Dr. Harold Goldthorpe, Department of Biochemistry.

[2] These studies were carried out by Dr. Hillman Castle, Department of Internal Medicine.

tardation, retarded sexual development, or signs of adrenocortical insufficiency. Although there was severe growth retardation in each of these children, they were too young to manifest retarded sexual development and there were no obvious signs of adrenocortical insufficiency.

The response of the serum PBI level to exogenous thyrotropic hormone has been employed to differentiate primary from secondary hypothyroidism. This test is usually combined with thyroidal I^{131} uptake studies made before and during the administration of the thyrotropic hormone (12) but there is indication that the former procedure is adequate in itself (13). In these patients no increase in the serum PBI level occurred following administration of thyrotropic hormone, indicating that their hypothyroidism was primary and not secondary.

Thus, hypothyroidism in these patients was due either 1) to a lack of thyroid tissue, or 2) to a failure of the end-organ to produce thyroid hormone. The absence of demonstrable goiter favored the former hypothesis. The results of radioiodine uptake studies were confirmatory and permitted a definitive diagnosis of athyreotic cretinism.

The occurrence of more than one instance of athyreotic cretinism in a family is rare (8). However, it was possible to establish this diagnosis in the 3 siblings whose data are presented here. Examination of their geneologic background revealed at least 12 additional closely related individuals who may have been similarly affected. This finding suggests that athyreosis in these children probably was due to a specific inherited defect in the gene.

SUMMARY

The unusual occurrence of athyreotic cretinism in 3 siblings is reported. The diagnosis of hypothyroidism was made by clinical observation and standard laboratory procedures. The diagnosis of the type of cretinism was established by 1) studies of radioiodine uptake, and 2) failure of the serum PBI level to respond to thyrotropic hormone administration.

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THYROTOXIC MYOPATHY AND MYASTHENIA GRAVIS: A CASE REPORT

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ALTHOUGH muscular weakness is characteristic of hyperthyroidism, the simultaneous occurrence of this condition and myasthenia gravis in the same patient is relatively rare (1–4). However, 6 per cent of patients at the Mayo Clinic with myasthenia gravis either had associated hyperthyroidism or had had this disease before the onset of myasthenia gravis (5).

In the Johns Hopkins series of 270 cases of ocular myasthenia gravis (220 generalized and 50 localized), 12 showed some abnormality of the thyroid gland; and of these, 8 had hyperthyroidism (6).

The myasthenia of chronic thyrotoxic myopathy superficially resembles myasthenia gravis and the two conditions may be easily confused, particularly when exophthalmic ophthalmoplegia is present (4). In 1908, Rennie (7, 8) published the first report of hyperthyroidism and associated myasthenia gravis. In 1946, Cohen (4) suggested that perhaps this was an instance of exophthalmic ophthalmoplegia and not of the combined diseases. Since then, several cases of this disease combination have been reported (1–4, 6–18). However, in several of these the diagnosis of myasthenia gravis can be questioned, particularly since the observation of McEachern and Ross (19) that thyrotoxic myopathy itself shows a temporary neostigmine response. The following case is reported as a well documented instance of the simultaneous appearance of these two conditions:

CASE REPORT

S.T., a 48-year-old white male, was admitted on October 7, 1948, because of diplopia of six months' duration and weight loss. He had been hospitalized in 1939 and in 1947 for active pulmonary tuberculosis.

On admission the skin was warm, dry and smooth; the hair was coarse and thick. There was bilateral exophthalmos, greater on the right, but no lid lag. There was paralysis of the right internal rectus, some weakness of the left internal rectus, mild weakness of the levators, insufficient power of convergence and a tendency to stare. The right lobe of the thyroid was enlarged. Auricular fibrillation was present. The remainder of the physical examination was unrevealing. The basal metabolic rate (BMR) was +69 per cent and the blood cholesterol level was 148 mg. per 100 ml. Other laboratory studies,

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including x-ray examination of the skull, gave normal findings. A chest roentgenogram revealed tuberculous infiltration and emphysema. The electrocardiogram showed auricular fibrillation with occasional ventricular ectopic beats.

A marked increase in the power of the extraocular muscles was elicted following injection of 2 mg. of neostigmine. A dose of 60 mg. daily by mouth provided adequate maintenance of this effect. The patient was started on *n*-propylthiouracil, 300 mg. daily, and was discharged. By March 1949, the patient was asymptomatic and was taking neostigmine, 150 mg. daily. The BMR was +6 per cent. The dosage of propylthiouracil was reduced to 100 mg. daily and he was maintained on this regimen until July 1950, when the BMR was +17 per cent; propylthiouracil was then increased to 150 mg. daily. He was maintained thus until January 1953, when he was found to have a mild recurrence of symptoms. There was cervical venous distention. Physical signs of thyrotoxicosis and myasthenia gravis were present. Blood cholesterol concentration was 233 mg. per 100 ml. and the BMR was +21 per cent. An electrocardiogram showed auricular fibrillation and chronic coronary insufficiency. Venous pressure was elevated.

On February 9, ten days after the withdrawal of propylthiouracil, an I^{131} tracer study indicated a euthyroid state. This study was repeated seven weeks after the withdrawal of propylthiouracil and revealed a characteristic hyperthyroid uptake of I^{131} by the thyroid (Fig. 1).

On April 17, 1953, the patient complained of generalized weakness. There was a

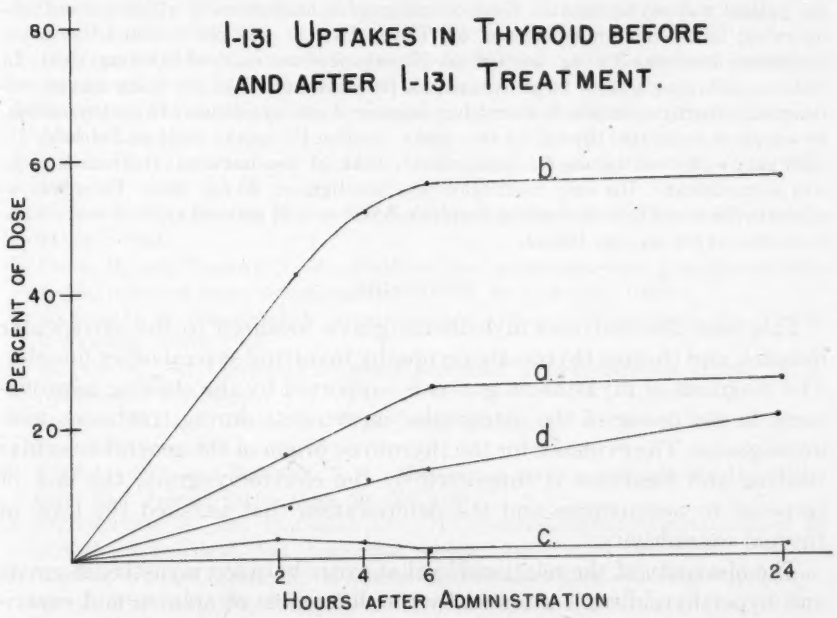

FIG. 1. S.T.'s pre-treatment I^{131} tracer curves: **a)** Feb. 9, 1953, ten days after withdrawal of propylthiouracil; **b)** March 23, seven weeks after withdrawal of propylthiouracil. Post-treatment curves: **c)** July 6, ten weeks, and **d)** Feb. 15, 1954, ten months, after therapeutic dose of 6 millicuries of I^{131}.

marked increase in his nervousness, irritability and sweating. He had lost 20 pounds, despite an excessive appetite. There was marked wasting of the sternomastoids, upper trapezii and the intrinsic muscles of the hands. There was moderate wasting of the muscles of the shoulder and pelvic girdles, the arms and legs. Extraocular movements were markedly limited. There was moderate weakness of the orbicularis oculi, flexors of the neck and the thighs, and extensors of the legs. There was also slight but definite weakness of the facial muscles, the abductors of the arms, and the flexors and extensors of the forearms, hands, lower legs and feet. The grips measured 20 Kg. (normal: 45–50 Kg.). There were no abnormal findings on neurologic examination. The BMR was +47 per cent.

An electromyogram, recorded from the abductor of the right fifth finger, showed no evidence of neuromuscular block. An injection of 1.25 mg. of neostigmine into the right brachial artery resulted in transient marked weakness of the injected extremity, which was followed by slight fasciculations in the injected extremity and by marked fasciculations in the muscles of the legs and the periorbital muscles. There was slight improvement in the ptosis and marked improvement in the extraocular movements, but no change in the orbicularis oculi nor in the muscles of the neck, shoulder, pelvic girdles and extremities. A therapeutic dose of 6 millicuries of radioiodine was administered and the patient was discharged shortly thereafter.

On June 17, the BMR was −12 per cent and the serum cholesterol level was 316 mg. per 100 ml. On July 6, the thyroidal I^{131} uptake was frankly hypothyroid. On August 6, the patient was asymptomatic. Electrocardiographic findings were within normal limits except for a sinus bradycardia of 54. The BMR was −21 per cent and the serum cholesterol level was 376 mg. per 100 ml. Neostigmine was reduced to 90 mg. daily. In October, following a fairly large hemoptysis, he was hospitalized but there was no evidence of active tuberculosis. In December, because of mild symptoms of hypothyroidism, he was given desiccated thyroid for two weeks. Another I^{131} uptake study on February 15, 1954 gave euthyroid values. On September 7, 1954, he was last seen, at which time he was asymptomatic. His only medication was neostigmine, 90 mg. daily. There was no clinical evidence of hypothyroidism despite a BMR of −22 per cent and a serum cholesterol level of 316 mg. per 100 ml.

DISCUSSION

This case demonstrates myasthenia gravis localized to the extraocular muscles, and chronic thyrotoxic myopathy involving several other muscles. The diagnosis of myasthenia gravis is supported by the striking improvement in the power of the extraocular movements during treatment with neostigmine. The evidence for the thyrotoxic origin of the general muscular wasting and weakness is supported by the electromyogram, the lack of response to neostigmine and the deterioration that paralled the level of thyroid overactivity.

The obscurity of the relationship that exists between myasthenia gravis and hyperthyroidism is attested by the differences of opinion and experience cited in the literature. Cases have been reported in which the severity of myasthenia gravis paralleled that of thyrotoxicosis (2, 3, 5, 6, 7, 10, 18) Other authors state that there is a reciprocal relationship between the severity of the myasthenia gravis and that of the thyrotoxicosis (1, 11, 16).

Surgical treatment of exophthalmic goiter complicated by myasthenia gravis offers difficulties (18). X-ray therapy, radioiodine, and thiouracil and its derivatives have been employed for the control of thyrotoxicosis. In the present case a single dose of 6 millicuries of I^{131} produced an excellent result despite a transient period of clinical hypothyroidism and persistent laboratory evidence of a mildly hypothyroid state.

SUMMARY

A case is reported of simultaneous hyperthyroidism, thyrotoxic myopathy and myasthenia gravis. Radioiodine therapy produced a remission of the hyperthyroidism and thyrotoxic myopathy but had no effect on the myasthenia gravis.

Acknowledgments

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THE INFLUENCE OF DIET ON URINARY AMINO NITROGEN LEVELS

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QUANTITATIVE and qualitative alterations in the urinary excretion of amino acids are now widely recognized as nonspecific findings in a great variety of physiologic and pathologic conditions. However, in most studies of urinary excretion, whether of total amino nitrogen or of amino acid patterns, interpretation of the results is made difficult by a lack of adequate control of the dietary protein intake.

The influence of dietary protein content on plasma and urinary nitrogen levels has been investigated, both in normal humans and in laboratory animals. Most of these investigations (1–4) indicate a direct relationship between the protein intake and the urinary amino nitrogen output. It would seem reasonable, therefore, that reports dealing with pathologic states should also take into account the nitrogen intake, in presenting what purport to be characteristic excretion levels or patterns of amino acids in specific disease states. Although some authors do observe this precaution (5, 6), most do not. In view of the importance attached to amino acid excretion levels as possible diagnostic or prognostic signs in some diseases, it seemed of value to compare quantitatively the effects of the dietary protein intake and of several pathologic states on the urinary amino nitrogen output. The results of the present study suggest that in most instances the influence of the diet is quantitatively as great as that of any other factor.

MATERIALS AND METHODS

One hundred and seven examinations were made of 24-hour urine specimens obtained from patients, physicians, and healthy medical students. The subjects examined were grouped according to diagnosis, as shown in the second column of Table 1. The diet given to each of the various groups is shown in the same table, together with the respective analytical results.

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The high-caloric, high-protein diets indicated for Groups 1 and 6 consisted essentially of the common basic diet received by Groups 2 and 3, with reduced fat content, and supplemented by the necessary amounts of first-class protein in the form of skim-milk cheese, lean meat, and milk.

Urine samples were collected in dark bottles, each containing 50 ml. of approximately 3N hydrochloric acid, and 1 ml. of a 10 per cent (w/v) solution of thymol in 95 per cent alcohol. The amino nitrogen content was measured by the method of Pope and Stevens (7) as modified by Albanese and Irby (8).

RESULTS

The 24-hour amino nitrogen output values given in Table 1 are the respective mean values and standard deviations for the corresponding groups.

TABLE 1. DAILY DIET AND URINARY AMINO NITROGEN EXCRETION OF GROUPS STUDIED

Group No.	Diagnosis	No. of assays	Daily caloric intake	Daily protein intake, (Gm.)	Urinary amino N (mg./24 hrs.)
1	Acute hepatitis	13	3,000–3,500	100	378 ± 56
2	Benign obstructive jaundice	11	2,500–3,000	60	195 ± 17
3	Non-hepatic diseases*	27	2,500–2,000	60	186 ± 14
4	Malignant neoplasms	10	0–2,500	0–60	271 ± 33
5	Glomerular lesions	6	2,000–2,500	30–60	142 ± 26
6	Non-hepatic diseases,* with diet for cirrhotic patients	11	3,000–3,500	140	307 ± 35
7	Normal controls	13	ad lib.	at least 100	349 ± 41

* Includes such conditions as failure-free cardiac disorders, infectious diseases in convalescence, rheumatic diseases, or hematologic diseases.

In addition to these groups, there were 2 cases of nephrotic syndrome, which were not included in the statistical comparison, because of the small number of cases. It is interesting to note, however, that of the 2 patients, the one with a protein intake of 60 Gm. daily had an amino nitrogen output of 354 mg., whereas the other, receiving 100 Gm. of protein, put out 684 mg. of amino nitrogen in twenty-four hours.

Fourteen determinations were also made on patients with portal cirrhosis, receiving a diet containing 3,000–3,500 calories and 140 Gm. of protein daily. The urinary amino nitrogen content was 254 ± 47 mg. per diem. This group is not included in the tables, for reasons explained subsequently.

Using the foregoing values, the results for the various groups were paired off in all possible combinations, and a statistical analysis was made of the differences in results for the members of each pair. For each such difference, the p value was obtained from the standard tables (9). All those differences for which the p value was less than 0.05 were considered statistically significant, and are listed in Table 2.

DISCUSSION

The various groups of cases shown in Table 1 can be sorted roughly into three levels of amino nitrogen output. On the highest level are the groups

TABLE 2. GROUPS SHOWING SIGNIFICANT DIFFERENCES IN MEAN VALUES OF URINARY AMINO NITROGEN OUTPUT

Groups under comparison	Respective mean values	Result of "t" test	P
Acute hepatitis; obstructive jaundice	378–195	3.12	0.01
Acute hepatitis; non-hepatic diseases	378–186	3.31	0.01
Acute hepatitis; glomerular lesions	378–142	3.79	0.01
Obstructive jaundice; normal subjects	195–349	3.49	0.01
Obstructive jaundice; non-hepatic diseases, with diet for cirrhotics	195–307	2.86	0.01
Obstructive jaundice; glomerular lesions	195–142	3.00	0.01
Non-hepatic diseases; normal subjects	186–349	3.77	0.01
Non-hepatic diseases; malignant neoplasms	186–271	2.34	0.05
Non-hepatic diseases; non-hepatic diseases, with diet for cirrhotics	186–307	3.27	0.01
Normal subjects; glomerular lesions	349–142	4.25	0.01
Malignant neoplasms; glomerular lesions	271–142	3.03	0.01
Non-hepatic diseases, with diet for cirrhotics; glomerular lesions	307–142	3.74	0.01

with acute hepatitis, non-hepatic diseases (cirrhosis diet), or malignant neoplasms, and the normal controls. On an intermediate level are the groups with non-hepatic diseases (ordinary ward diet), and those with benign obstructive jaundice. On the lowest excretion level are the patients with glomerular lesions. It may be seen from Table 2 that, with very few exceptions, there is a statistically significant difference between the mean values for any two groups at different levels of amino nitrogen excretion.

If, for the moment, we ignore the group with malignant neoplasms, we find that the only feature common to all the groups at a given level of amino nitrogen excretion, is the corresponding level of protein taken in the diet. The difference between the average excretory levels for the group of non-hepatic cases on an intake of 60 Gm. of protein, and for the group

of similar cases receiving 140 Gm. of protein daily, is obvious. Similarly, there is a clear-cut difference between the results of the acute hepatitis group and of the normal controls, on the one hand, and those of the non-hepatic disease group and the benign obstructive jaundice group, on the other. Here again, the former two groups had a diet with a protein content of 100 Gm. or more daily, whereas the latter two received 60 Gm. of protein daily.

In the group with glomerular lesions (acute or chronic glomerulonephritis), only 1 patient had nitrogen retention, so that it is difficult to attribute the decreased amino nitrogen excretion to reduced glomerular filtration. It seems more reasonable to consider their reduced protein intake (30 Gm. daily in most cases) as the principal factor. It would be of interest to know what influence their restricted salt intake might have, with respect to the low amino nitrogen excretion.

Unfortunately, the results for the cirrhotic patients could not be compared with those of the other groups, as their generally poor state, with anorexia, resulted in varied and incomplete consumption of the diet supplied to them. However, it is evident that the urinary amino nitrogen level, under these circumstances, was not clearly elevated by reason of the hepatic cirrhosis per se.

In contrast to all the others, the group with cancer had a very low caloric and protein intake, because of either terminal anorexia or esophageal obstruction. Therefore their amino nitrogen excretion depended principally upon destruction of their own tissue proteins, under the influence of their malignant lesions. Thus they present a striking comparison of the relative influences of this pathologic process and of dietary protein intake, on the urinary amino nitrogen excretion.

A further comparison of interest is offered by the patients with acute hepatitis, all of whom were jaundiced at the time of the examination. These patients, on a diet essentially similar to that of the normal controls, showed a somewhat (though not significantly) higher excretion of amino nitrogen than the latter. The pathologic process of acute hepatitis might be expected to increase the output, either by autolysis of liver protein or by impairment of the deaminating capacity of the liver cells. It is noteworthy that this group showed the highest mean value of all, and that the highest value within this group (597 mg. in twenty-four hours) was found in a case at the peak of its intensity. The tremendous accumulation of amino acids in the blood in a fatal case of acute yellow atrophy of the liver reported by Rabinowitch (10) gives some indication of the effect that this factor might conceivably have upon the amino nitrogen excretion in extreme cases; but in acute hepatitis of average intensity, such as these cases, the effect of the pathologic process seems to be less than that of the dietary protein intake.

Recently some observers have reported no apparent influence of dietary protein content on urinary amino acid levels, in contrast to the findings described here. The measurements, however, are not strictly comparable with those in the present paper. Wharton and Patton (11) found no correlation between protein intake and urinary excretion of essential amino acids, in normal women receiving 150 Gm. and 200 Gm. of protein daily. Both of these figures represent very high protein intakes, rather than high and low values as in the present study. In addition, their subjects showed positive nitrogen balances of magnitudes proportional to their protein intakes, and therefore could not have been equilibrated on their respective diets. Finally, their results refer to the ten essential amino acids, and not to total α-amino nitrogen. Eckhardt and Davidson, in an earlier study (3), showed a much clearer influence of the dietary protein level on the urinary content of total α-amino nitrogen than on the excretions of certain individual amino acids.

Similarly, Gabuzda, Eckhardt and Davidson (12) reported an apparent failure of dietary protein content to influence urinary excretion of essential amino acids in cirrhotic patients. Their results, however, indicate a clear-cut effect of alteration of protein intake in their normal control and in 2 of their 4 cirrhotics, whereas in the other 2 cirrhotics there were only insignificant increases in essential amino acid excretion following increase in protein intake. Their results, therefore, appear to give somewhat more evidence for, than against a significant effect of dietary protein intake on urinary amino acid excretion. Again, it should be noted that they measured the ten essential amino acids rather than total amino nitrogen.

In view of the foregoing findings, it seems reasonable that any conclusions concerning amino acid metabolism in various disease states, and any diagnostic value attributed to the level of excretion in such conditions, can only be interpreted in the light of the dietary protein intake. Although one investigator might find an abnormality of amino acid excretion in patients with a certain disease, another might be unable to confirm these findings if the conditions of diet were not the same. Therefore it is urged that reports concerning variations in amino acid metabolism be accompanied by an explicit statement of the diet.

SUMMARY

The urinary amino nitrogen excretion has been studied in several groups of normal and pathologic subjects receiving various diets, and an attempt made to assess the relative influence of some of the factors possibly affecting the excretion levels. The findings suggest that variations in dietary protein intake are quantitatively as important as the presence or absence of pathologic processes, in determining these values. Attention is drawn

to the importance of this finding with respect to the interpretation of metabolic variations encountered in disease states.

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USE OF SUBLINGUAL ERGOT ALKALOIDS (HYDERGINE) IN THE CONTROL OF VASOMOTOR FLUSHES*

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SYMPTOMS of the menopause or "climacterium" occur in many women and may be present to a distressing degree of severity. Novak and Novak (1) state that at least 50 per cent of women cease menstruating between the ages of 45 and 50; 25 per cent before the age of 45; and 25 per cent at the age of 50 or beyond. The symptoms are many and varied, but most characteristic are the vasomotor flushes or "hot flashes." These may involve chiefly the head, neck and upper thorax, or may spread over the whole body with associated sweating, nervousness, anxiety and headaches. Although these flushes sometimes terminate spontaneously in a few months, in some patients they persist for many years and occasionally women aged 60 or more still complain of them, even though they may have been taking estrogens for ten or fifteen years.

Treatment with estrogens usually is specific and gives dramatic relief. It also causes a reversal of the physiologic changes characteristic of premenopausal years. However, in some patients there are definite contraindications to the use of estrogens. These include: a family history of carcinoma of the breast or genital tract, x-ray or surgical castration because of breast or genital-tract carcinoma, and breast dyscrasias in the menopausal patient (2–6). In other instances, adequate estrogen therapy may cause escape bleeding. Then there are those patients in whom the customary cyclic interruption of therapy for one week each month may result in periodic recurrence of distressing symptoms or withdrawal bleeding. Androgens are occasionally helpful, but if given alone do not have comparable effectiveness and may have distressing side reactions. Any agent that will aid these patients in the control of symptoms would, therefore, be helpful.

Although theories concerning the causation of vasomotor flushes vary, there seems no doubt that these flushes occur as a result of an instability of

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the vegetative nervous system. Therefore sedatives, including barbiturates, are occasionally helpful. In this study it was decided to evaluate the effects of hydrogenated ergot alkaloids (Hydergine) because of the known action on the vegetative nervous system. This agent has been used for the treatment of hypertensive (7) and vasomotor headaches (8), and prophylactically in the treatment of migraine (9). Sublingual tablets, each containing 0.5 mg. of hydrogenated ergot alkaloids, were used in this study, because of the recent demonstration of effectiveness of this mode of administration of alkaloids and steroids (10).

METHOD OF STUDY

Forty patients from private and clinic practice were treated. They were divided into five groups:

1. Twenty-six patients receiving cyclic therapy with estrogens or estrogen-like compounds, who complained of recurrence of flashes during the withdrawal period of one week each month.
2. Three patients castrated because of breast carcinoma and suffering from severe flashes.
3. Six patients castrated by irradiation for other reasons, and who also had breast dyscrasias.
4. Two patients with a surgical menopause and flashes.
5. Three patients receiving estrogens, who experienced escape bleeding and flashes.

The initial dosage was two tablets four times daily. One tablet was placed in each buccal gutter and then dissolved. A few patients complained of the sweet taste, but otherwise no unpleasant effects were noted. If the flashes were satisfactorily controlled, the dose was gradually reduced and in some patients a dosage of one or two tablets daily was sufficient for maintenance therapy.

RESULTS

The results are indicated in Table 1. All but 3 patients obtained some relief. In 20 women in whom the results were "good," placebos were used alternately; 12 of these noted a return of symptoms, 6 had only partial relief, and 2 had results which were as satisfactory as with medication. Illustrative cases are given in the Appendix.

DISCUSSION

Patients with innate or acquired instability of the autonomic system appear to be most liable to have severe symptoms at the menopause. However, it seems logical to treat the imbalance of an oversensitive vegetative system with a preparation known to have a specific, but non-depressing

TABLE 1. RESULTS OF TREATMENT WITH SUBLINGUAL HYDROGENATED ERGOT ALKALOIDS

No. of cases	Clinical condition	Excellent	Good	Partial relief	Poor	Toxicity
26	Functional disorders of menopause, with flashes	—	16	8	2	None
3	Castrated for breast carcinoma; flashes	—	—	3	—	None
6	Breast dyscrasias; castrated; flashes	—	4	1	1	None
2	Surgical menopause; flashes	2	—	—	—	None
3	Escape bleeding; with flashes	—	2	1	—	None
—		—	—	—	—	
40	*Totals*	*2*	*22*	*13*	*3*	

effect on this portion of the nervous system. Hydergine is a combination of three hydrogenated ergot alkaloids, dihydroergocornine, dihydroergocristine and dihydrokryptine. Rothlin (11) demonstrated that hydrogenation decreased the toxicity of the natural alkaloids of ergot and increased the sympathicolytic and adrenolytic effects. Bircher and Cerletti (12) showed that Hydergine possesses marked sympathicolytic and adrenolytic effects, as well as a central vasodilating action which is due to either an inhibition of the vasoconstrictor center or to stimulation of the vasodilator center.

The results reported would seem to indicate that these pharmacologic actions are frequently effective in blocking the impulses which lead to vasomotor flushes. The mode of action is not entirely clear, but this is true also concerning the mode of production of menopausal symptoms. Although the results are not invariably predictable, they are occasionally dramatic and would seem to indicate that this preparation is frequently an effective therapeutic tool in controlling flushes when an estrogen cannot be used.

SUMMARY

The autonomic nervous system seems to play an important role in the causation of vasomotor flushes in climacteric patients. Estrogens usually control these symptoms adequately, but occasionally are contraindicated. Hydrogenated ergot alkaloids (Hydergine) were used in 40 women complaining of severe flashes. They were given two sublingual tablets, each containing 0.5 mg., four times daily. Of these patients, 2 experienced "excellent" relief, 22 "good" relief, 13 partial relief, and 3 no relief. Placebo tablets were given alternately in the group of 22 patients with "good" re-

sponse, resulting in a recurrence of symptoms in 12, and a regression to partial relief in 6. This preparation of hydrogenated ergot alkaloids is therefore considered useful in the control of flushes and sweats in patients suffering from climacteric symptoms.

APPENDIX

Illustrative Cases

No. 1, E. H., aged 29, had a left oophorectomy at the age of 23. A right ovarian resection was performed when she was 26 years old. Menstruation occurred thereafter at intervals of thirty to one hundred and eighty days. She had amenorrhea of four months' duration when first seen. There were nocturnal flushes as well as 3 to 4 flushes during the day. With Hydergine, one tablet twice daily, the results were poor. Dosage was increased to two tablets four times a day, and there was 100 per cent relief. During substitution of placebos there was a return of symptoms within forty-eight hours. Control was again effected with the same dosage of Hydergine. No sedative effect was noted.

No. 2, A. S., aged 50, was a registered nurse with a family history of breast carcinoma. She had chronic cystic mastitis. Vasomotor flushes were partially relieved with various sedatives, including phenobarbital. Results were good with Hydergine, two tablets three times a day. During placebo substitution on two occasions, there was partial relief—about the same as that with phenobarbital. The patient is now taking Hydergine twice daily, with good results. There has been no sedative effect.

No. 3, R. T., aged 46, had amenorrhea for the preceding two years, except for withdrawal bleeding when estrogen therapy was omitted on two occasions. Flashes of moderately severe intensity (4 to 6 during the day and also at night) were well controlled with sodium estrone sulfate and stilbestrol. Hydergine, two tablets four times daily, eliminated about 75 per cent of the flashes, but control was not as complete as with estrogen or stilbestrol. There was no sedative action.

No. 4, J. C., aged 45, had irregular menses during the preceding year, and flushes for the preceding four to five months. These were moderately severe and mostly nocturnal. Sodium estrone sulfate was started in a dose of 1.25 mg. daily for three weeks, with one week of rest. Symptoms returned during withdrawal. Hydergine (one tablet three times a day), which was substituted during the withdrawal period, gave partial relief. No sedation was noted.

No. 5, M. E., aged 42, was treated by radical mastectomy and x-ray castration for carcinoma of the breast. Thereafter she had severe flushes and sweats (8 to 12 daily), with marked nocturnal flashes. Hydergine, two tablets four times a day, gave partial relief for three months. A dosage of one tablet three times a day also gave partial relief.

No. 6, E. K., aged 49, had been taking dienestrol and sodium estrone sulfate cyclically with results ranging from fair to good. She had been treated for psychosis with shock therapy. After another recurrence of psychotic behavior and subsequent therapy, there was a return of severe flashes, in spite of estrogen therapy. Hydergine failed to give any relief. Hexestrol, however, gave marked relief of symptoms.

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Teaching Clinic

PITUITARY INSUFFICIENCY: CHARACTERISTIC CLINICAL AND AUTOPSY FINDINGS IN A PATIENT WITH PITUITARY MYXEDEMA

THE dominating effect of the anterior pituitary on the function of various other endocrine glands results in some diversity in the clinical picture encountered in hypopituitarism. Factors to be considered are: 1) the severity of damage to the pituitary; 2) the extent of diminished function of the glands secondarily affected, and 3) the type of response of the end-organs to the deficiency. In clinical experience, secondary hypothyroidism, secondary hypoadrenocorticism and secondary hypogonadism are encountered in varying degrees; one or all may be present, but occasionally manifestations of a single deficiency may predominate in the clinical findings. Thus, occasionally secondary hypothyroidism is most conspicuous, resulting in the picture of "pituitary myxedema" as described by Means, Hertz, and Lerman in 1940 (1). The case presented here is one of pituitary myxedema followed for several years. The patient finally died, and an autopsy provided the opportunity to examine his endocrine glands. Study of the case demonstrates several points of interest regarding hypopituitarism.

CASE REPORT

H.C., (U132003), a man aged 52 years, was first seen at the University of California Clinic on August 21, 1946; his chief complaints were anemia and weakness. He had been a welder, and had been in good health until five years before, when he first noted thinning and loss of hair from head and body, followed by diminution and finally complete loss of libido and sexual potency. His features had become somewhat puffy, and treatment with thyroid had been tried for a short time with only slight, questionable benefit. A decrease in weight from a level of 165 pounds to 145 pounds had occurred two years before. At that time he had had a dental extraction which was complicated by a hemorrhage from the socket, of such severity that hospitalization was required for control. Following this, his hemoglobin was 62 per cent, and he was troubled by weakness, drowsiness, and a sensation of coldness. His weight continued to drop, and one year before his initial visit had decreased another 26 pounds to a level of 119 pounds. Treatment with iron and liver (the latter orally and parenterally) had not improved the moderately severe anemia. In the preceding year there had been some improvement in appetite, and he had regained 20 to 25 pounds. However, the other symptoms continued and progressed so that he was no longer able to work. In appearance he had aged considerably. The beard growth, which formerly had been vigorous, was now decreased so that shaving was necessary only about every three days.

On *physical examination* (Fig. 1), his height was 5 feet, 7¼ inches, and weight 143 pounds. He appeared considerably older than his stated age of 52. His face was puffy, his complexion was sallow and pale, and his skin cold and dry. The hair was fine in texture, thin on the head, absent in the axillae, and scant in the pubic area. Speech was slow and slurred, and the voice was low pitched. The pulse rate was 65 per minute, and blood pressure 80/60 mm. of Hg. The penis was normal in size, but the testes were soft and atrophic.

Laboratory findings were as follows: basal metabolic rate, −47 per cent; plasma cholesterol level, 183 mg. per 100 ml.; serum protein-bound iodine level, 4.5 micrograms per 100 ml.; blood carotin absent (indicating no increase); icterus index, 9; hemoglobin, 68 per cent (9.3 Gm. per 100 ml.) (later 60 per cent); red blood cells, 3,200,000 and white blood cells, 5,900 per cu. mm. Gastric analysis showed no free acid and a reduced amount of total acid. The results of sternal puncture were interpreted as showing a normochromic anemia. The 17-ketosteroid excretion values were 2.9 and 3.5 mg. per twenty-four hours (ketonic fraction). No FSH was detected in the urine at the lowest level tested—6.2 mouse units per twenty-four hours. A glucose tolerance test (Folin and Wu method) after 100 Gm. of glucose orally showed the following concentrations of blood sugar: fasting, 76 mg. per 100 ml.; half hour, 98 mg.; one hour, 134 mg.; two hours, 123 mg.; three hours 81 mg.; four hours, 73 mg.; five hours, 54 mg.; and six hours, 43 mg. per 100 ml. At the end of the test the patient noted some weakness and vertigo. An insulin tolerance test, using 3 units of regular insulin intravenously, showed: fasting blood sugar, 63 mg. per

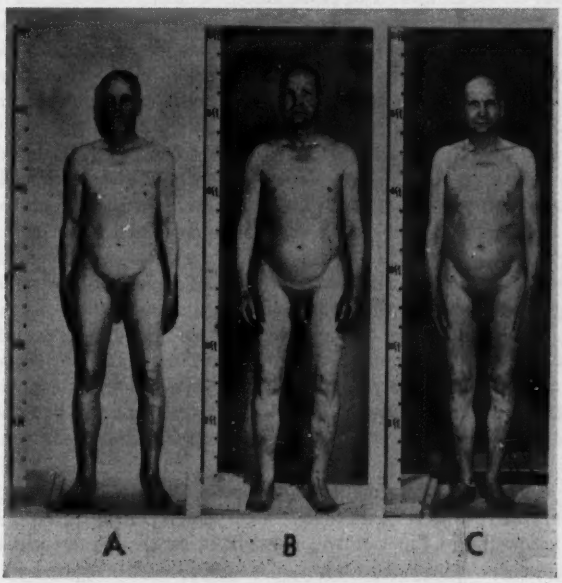

FIG. 1. **A.** September 19, 1949. Appearance of patient before treatment; age 52 years, weight 143 pounds. **B.** Appearance after eleven months of treatment with methyltestosterone. Note improved nutritional state, but obvious puffiness and edema; weight 170 pounds. **C.** November 22, 1948. Appearance after one year of treatment with methyltestosterone linguets plus thyroid; weight 150 pounds.

100 ml.; half hour, 31 mg.; one hour, 28 mg.; and one and a half hours, 26 mg. per 100 ml. At this point the patient became confused and had a convulsion; the test was terminated and 20 cc. of 50 per cent glucose was administered intravenously, whereupon the patient revived promptly. An electrocardiogram showed bradycardia (rate 52), low voltage, slight depression of S T-1, 2, and 3, and diphasic T-1, 2 and 4. X-ray examination showed the sella turcica to be normal.

Treatment with desiccated thyroid was tried first, starting at .03 Gm. (½ grain) daily. In ten days the patient felt no differently, but his weight had fallen from 143 to 139 pounds, so it was decided to discontinue thyroid rather than to try a larger dose. Methyltestosterone linguets were then started (November 14, 1946) in a dosage of 10 mg. daily, absorbed buccally. The results were dramatic; in two weeks his strength was improved, erections had occurred, he felt less cold, and his appetite was better. His skin and hair were more normal, and an increased amount of hair was noted on his arms. He felt that his mental reactions were quicker and memory was better. After three months' treatment the patient had gained 4 pounds, blood pressure had risen to 125/70 (originally 80/60), hemoglobin had increased to 70 per cent and red blood cells to 3,700,000 per cu. mm. All of this had occurred with methyltestosterone as the only therapy; no other endocrine medication, iron or vitamins had been administered.

The dosage of methyltestosterone linguets then was increased to 20 mg. daily. Improvement continued, but the weight increased rapidly. Some of the increase was obviously due to fluid retention and edema, causing a puffy appearance of the face (Fig. 1B) and swelling of the ankles. The basal metabolic rate, however, was still low (−39 per cent), and the electrocardiogram showed little change. After three months the dosage of methyltestosterone linguets was cut to 15 mg. daily, then to 10, and finally to 5 mg. daily. There was some decrease in edema but considerable puffiness continued; the weight remained at 170 pounds (143 when first seen). Otherwise the generally improved state had persisted.

After one year of treatment with methyltestosterone alone, it was decided to add desiccated thyroid to the regimen. On November 20, 1947, thyroid was started in a dosage of .03 Gm. (½ grain) daily, and after two weeks was increased to twice daily. Further improvement resulted, with loss of edema and puffiness, and disappearance of leg cramps which had become troublesome. The thyroid dosage later was increased to .065 Gm. (1 grain) twice daily; this was followed by increased rapidity of movement and speech and by more general alertness. After three months the basal metabolic rate had risen to −23 per cent, the plasma cholesterol level had dropped to 106 mg. per 100 ml. and that of serum protein-bound iodine had increased to 5.9 micrograms per 100 ml. The weight had fallen to a level of 150–155 pounds, at which level it became stabilized. The pulse rate was 80 and the blood pressure 110/60. Erections occurred two or three times a week, and there was a heavier growth of the beard, so that he had to shave every second or third day.

This routine of medication (methyltestosterone linguets, 5 mg., and thyroid, 0.12 Gm. (2 grains) daily) was continued for three years. During this period the patient continued to feel well. Sex function was manifested by the occurrence of erections two or three times weekly. The pulse rate was 70 to 80; blood pressure was 90–110/60–70. Blood counts remained normal, and the hemoglobin level rose from 11.2 to 13.9 Gm. per 100 ml. Basal metabolic rates were −13, −11, and −14 per cent. Plasma cholesterol levels were 163, 206, and 218 mg. per 100 ml. Values for 17-ketosteroid excretion were: less than 1 mg., 2.2 mg., and 7.8 mg. per twenty-four hours. Assay of the urine for FSH gave negative findings at 5 mouse units and later at 3 mouse units. Glucose tolerance

tests showed slightly higher blood sugar curves which still dipped to moderately hypoglycemic levels at five and six hours.

In January 1951, because of persistently low blood pressure (under 100 mg. of Hg systolic) it was decided to add desoxycorticosterone to the patient's regimen. This was prescribed as 5-mg. buccal tablets, taken once, and later twice daily. Some further improvement in general strength resulted, and the blood pressure ranged from 100 to 120 systolic and 60 to 70 diastolic. The weight, however, continued to decrease, reaching 130 pounds in August 1952.

Laboratory results at that time included a hemoglobin level of 13.1 Gm. per 100 ml.; red blood cell count, 4,230,000 per cu. mm.; basal metabolic rate, −8 per cent; plasma cholesterol concentration, 173 mg. per 100 ml.; serum protein-bound iodine concentration, 4.6 micrograms per 100 ml.; urinary 17-ketosteroids, 2 mg. per twenty-four hours; urinary FSH assay, negative at 5 mouse units; glucose tolerance test (Somogyi method), fasting blood sugar level, 59 mg. per 100 ml.; half hour, 112 mg.; one hour, 160 mg.; two hours, 168 mg.; three hours, 124 mg.; four hours, 45 mg.; five hours, 35 mg.; and six hours, 50 mg. per 100 ml. The electrocardiogram showed a partial A–V block.

Four months later, while at home, the patient was noted to act "strangely," be less responsive, and spend most of the time in bed. After three or four days he could not be aroused in the morning, and his arms and legs were stiff and "drawn up." There was some incoherent speech and "thrashing about." He was sent into the hospital, where his pulse rate was found to be 88, and his blood pressure 150/70; reflexes were hyperactive, and Babinski reflexes were present bilaterally. Supportive therapy was started, but the patient died suddenly before any further treatment or studies could be carried out.

Autopsy was performed. The following are the pertinent pathologic findings:

The *pituitary* was very small and soft in consistency. Microscopically, there was loss of structure, with only scattered islets of degenerated cells (Fig. 2).

The *thyroid* was represented by two oval masses weighing 11 grams. On section, there were scanty alveoli and groups of exhaustion cells (Fig. 3). Extensive fibrosis was present, with round-cell infiltration typical of thyroiditis.

The *adrenals* appeared atrophic and scarred; the zona glomerulosa and zona reticularis were especially thin (Fig. 4), and the medulla was fibrotic. The adrenals weighed 3.5 and 2.5 grams, respectively.

The *testes* were small, weighing 5 and 5.5 grams. Sections showed atrophy and fibrosis of the tubules, and thickening of the basement membranes and interstitial tissues. Only rare Leydig cells were seen (Fig. 5).

Other pathologic findings included tuberculosis of the cervical lymph nodes, emphysema, chronic pyelonephritis, arteriosclerosis of the coronary arteries, and nephrosclerosis. The brain showed no fresh vascular lesions and the results of examination were essentially negative except for some perivascular round-cell infiltration.

DISCUSSION

Clinical facts characteristic of hypopituitarism are emphasized by a study of the following items in this case:

1. *Change in weight:* This patient lost a considerable amount of weight in the early course of the disease, and then underwent partial recovery associated with some improvement in appetite. He never actually became cachectic, so the designation "pituitary cachexia" was not justified. Some change in weight frequently accompanies severe pituitary disease but, as

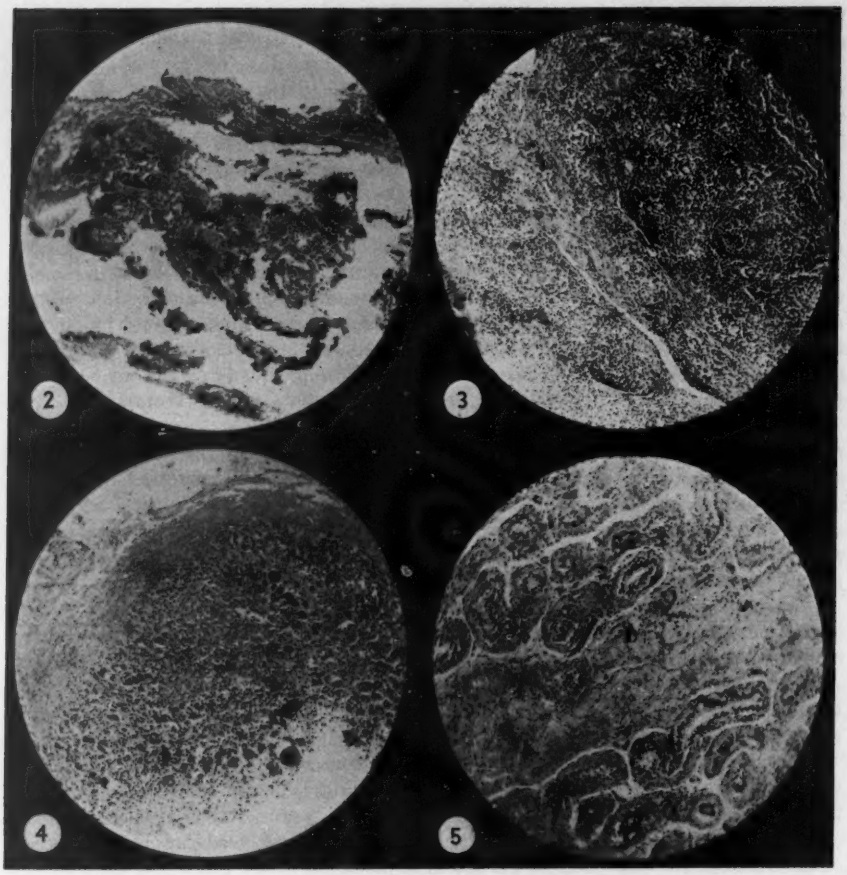

FIG. 2. Microphotograph of pituitary, showing entire width of pituitary remnant. Note nest of anterior-lobe cells.

FIG. 3. Microphotograph of thyroid. Note atrophic appearance with considerable round-cell infiltration.

FIG. 4. Microphotograph of adrenal, showing entire thickness of cortex. Note extreme atrophy.

FIG. 5. Microphotograph of testis. Note tubular atrophy, thickening of basement membrane and interstitital fibrosis.

pointed out by Sheehan and Summers (2) and also by Perkins and Rynearson (3), occasionally these patients do not lose weight; indeed a few, particularly the type classified as "pituitary myxedema," may actually gain, despite severe hypopituitarism. However, some change in weight is frequent with severe pituitary disease and a large percentage of such patients actually lose, even though relatively few progress to outright cachexia. The

general state of health as reflected by appetite and amount of food ingested may be definite factors in the weight fluctuations; but there are endogenous factors which are still poorly understood.

2. *Myxedematous appearance; treatment:* Even though this patient's appearance strongly suggested myxedema, thyroid medication alone did not improve the over-all clinical condition, either in the earlier course of the disease or later when he was under our observation. However, it was definitely beneficial when combined with methyltestosterone. This reaction is characteristic of pituitary myxedema. It must be emphasized that thyroid alone can actually be dangerous in severe hypopituitarism and may induce adrenocortical insufficiency, occasionally with crisis. However, many of these patients are given thyroid before the pituitary causation of the disease is recognized. If a patient with apparent myxedema responds poorly to thyroid, it should alert the clinician to the possibility of primary hypopituitarism. Other clues are early loss of sexual function and loss of sexual hair, both of which occurred in this case. Also, the occasional finding of a normal plasma cholesterol level in association with an appearance of myxedema (as in our patient) may be suggestive; in true thyroid myxedema the blood cholesterol level is elevated. More recently, with the availability of cortisone, the use of 5 to 20 mg. of cortisone acetate daily in combination with the thyroid medication adds a definite safety factor.

3. *Secondary hypoadrenocorticism:* This was not prominent at first in this patient, but developed gradually. Thus, treatment with desoxycorticosterone was helpful late in the course of the disease. This illustrates the point that these patients must be followed carefully, as occasionally the indications for treatment may change. On consideration of the microscopic appearance of the adrenals, it is surprising that symptoms and signs of adrenocortical insufficiency were not more prominent. Since no serious lesions of the central nervous system were found at autopsy, it is quite possible that this insufficiency, probably complicated by hypoglycemia, was a factor in the patient's eventual demise.

4. *Presence of carotinemia:* Carotinemia is found with great frequency in untreated myxedema, and it is of interest that, in spite of the sallow rather yellowish color of this patient's skin, no carotin was noted in his blood. This, too, may be a helpful point in distinguishing pituitary myxedema from primary thyroid myxedema.

5. *Other differential points:* If the condition appears in a woman following pregnancy, especially if it is complicated by hemorrhage and shock, primary hypopituitarism is most likely (Sheehan's syndrome). Other findings which suggest pituitary causation are x-ray evidence of a lesion in or about the sella turcica and marked sensitivity to insulin, as can be demonstrated by an insulin tolerance test. As in this patient, the test may induce

severe hypoglycemia, bringing on an emergency requiring termination of the test and prompt administration of intravenous glucose. It is usually safer to use half the recommended dose of insulin if hypopituitarism is suspected (0.05 unit instead of 0.1 unit of regular insulin per Kg. of body weight).

If the equipment is available, the effect of TSH (thyroid-stimulating hormone) on the thyroidal uptake of I^{131} can be investigated, as another differential point. Perloff, Levy and Despopoulos (4) have reported that following three consecutive daily injections of 20 to 30 mg. of thyroid-stimulating hormone (TSH), the thyroidal uptake of I^{131} (50-microcurie dose) was increased very little, if at all, in primary hypothyroid myxedema, whereas in pituitary myxedema it was definitely stimulated, the mean increase being 32.2 per cent.

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Editorial

DIABETIC NEPHROPATHY

IN RECENT years there has been an increasing recognition of the relative frequency and importance of renal disease in diabetes. In 1936 Kimmelstiel and Wilson (1) called particular attention to intercapillary lesions seen at postmortem examination in the kidneys of older diabetic patients. It is now common to refer to the clinical manifestations of this type of renal disease as the "Kimmelsteil-Wilson syndrome."

The experience of many workers amply confirms the high incidence of renal disease in patients with diabetes of long duration. However, there are certain features which deserve special comment: (a) Intercapillary glomerulosclerosis rarely, if ever, occurs as an isolated lesion and often seems relatively unimportant or may even be lacking. The renal involvement is almost invariably of mixed etiology, including both degenerative and infectious elements. This finding has been so striking that it has seemed preferable to designate the clinical syndrome as "mixed nephropathy associated with diabetes" or, more concisely, "diabetic nephropathy," rather than to ascribe the entire clinical picture to glomerulosclerosis alone. (b) Although diabetic nephropathy of mixed etiology is common in older patients, it occurs at all ages and is extraordinarily frequent in patients with onset of diabetes in the first three decades of life. (c) The occurrence of nephropathy appears directly related to inadequate control of diabetes over a period of many years (2).

The chronology of events in a typical case of diabetic nephropathy in the age group mentioned is remarkably uniform. A child, perhaps in the early teens, is found to have diabetes and the condition is brought under control by treatment with diet and insulin. Strict compliance with instructions often is maintained by the child's parents during the first months or years. Gradually, however, carelessness creeps into the management and as the years pass there is gross neglect, particularly beyond the age of 15 or 16 when the child has less parental supervision. There may be one or more episodes of diabetic coma, from which the patient is rescued, perhaps with relatively little difficulty. After ten to fifteen years of such inadequate treatment the patient, now probably is his late twenties or in his early thirties, is found to have proteinuria during one of his infrequent visits to the physician. Physical examination reveals a few scattered retinal micro-

aneurysms or hemorrhages. Although disturbed by these new developments, if he is informed of them, the patient may not become concerned enough to improve his inadequate management. In succeeding months and years, other signs and symptoms appear. The patient may note some diminution of vision, proteinuria increases and peripheral edema may become an annoying manifestation. Progressive hypertension may develop and there may be slight azotemia. In roentgenograms calcification may be seen within the arteries of the legs and pelvis. In time, typical diabetic retinopathy develops with hemorrhages, exudates and finally retinitis proliferans. At one point or another during the diabetic life, signs and symptoms of diabetic neuropathy, particularly peripheral neuritis, may have further complicated the situation. Despite the now frantic efforts to control the diabetes, no significant or lasting improvement in the kidney condition can be effected. Treatment by restriction of activity, a low-sodium diet, adjustments in the protein intake, ammonium chloride and mercurial diuretics, brings about only temporary improvement. Renal insufficiency progresses with massive proteinuria leading eventually to hypoproteinemia, reversal of the A/G ratio, anemia (resistant to treatment with iron) and constant azotemia. The terminal weeks or months are characterized by persistent hypertension, uremia and, often, cardiac decompensation and blindness or near-blindness. Not infrequently the insulin requirement falls terminally, due chiefly to inanition and markedly decreased food intake but possibly also to less well defined factors. Death takes place most frequently from uremia or congestive heart failure, or less commonly from myocardial infarction.

The incidence of a course such as that just sketched is alarmingly high in patients with onset of diabetes in childhood and adolescence. In this group it now accounts for more deaths than all other causes. The increasing menace of nephropathy is shown vividly in the accompanying table. This growing prominence of renal disease has been concomitant with the spectacular increase in recent years in the life expectancy of young persons with diabetes. Those patients who formerly died early in life of coma or acute infections are now avoiding or surviving these hazards by the use of insulin and antibiotics, only to succumb years later to complications reflecting degenerative vascular disease. Although the vascular disease in general manifests itself throughout the entire body, the resulting functional impairment is often localized predominantly to a single organ or site. Such is the case among these young diabetics who live for months or years with gradually increasing evidence of vascular disease which, though generalized, impairs renal function most severely.

Histologic examination of the kidneys of patients dying with diabetic nephropathy usually shows the lesion of intercapillary glomerulosclerosis.

TABLE 1. CAUSES OF DEATH IN 600 PATIENTS WITH ONSET OF DIABETES UNDER THE AGE OF 15 YEARS*

Period	1898 1922	1922 1936	1937 1943	1944 1949	1950 1953
No. of patients dying	157	99	81	144	119
Cause of death (%)					
Diabetic coma	86.0	56.0	27.2	9.7	6.7
Coronary heart disease	—	—	2.5	10.4	5.9
Nephropathy	—	2.0	12.3	36.8	63.0
Cerebro-vascular accident	—	—	1.2	2.1	5.9
Infections†	6.4	23.2	24.7	6.9	8.4
Tuberculosis	—	8.1	9.9	11.1	1.7
Other	7.6	10.7	22.2	23.0	8.4

* Compiled by Statistical Department, Metropolitan Life Insurance Company, from experience of Joslin Clinic. Adapted from table prepared by author and published in *Maryland State Med. J.* **3**: 8–13 (Jan.) 1954.

† Excluding tuberculosis.

However, in the usual case, this is far from the whole story. Other clear-cut lesions are present, including those of arteriosclerosis, arteriolarsclerosis, and acute and chronic pyelonephritis.

Of basic importance is the question of the cause of degenerative vascular disease, and in particular nephropathy, as seen in long-term diabetes. In the present state of knowledge, one must be content with the indefinite statement that the renal disease is related to the disordered metabolism of diabetes. Then comes the question: Is nephropathy related to the degree of control of diabetes over the years or is it simply a function of the duration of diabetes? In our group, we are convinced that there is a definite relationship between the degree of control and the occurrence of nephropathy. Duration of diabetes is important only because it allows a longer period of time for the deleterious forces arising from inadequate control to be operative. This conviction arose during a detailed and long-term study of 451 patients with onset of diabetes under the age of 30 years and with duration of the disease from ten to thirty-six years (3). The study included the collection of two types of data: (a) detailed information and concrete evidence as to the degree of control over the years, and (b) careful examination of the patients from various points of view to discover complications in the eyes, kidneys, heart, arteries and nervous sytem. The degrees of control were designated as "excellent," "good," "fair" and "poor" according to criteria published elsewhere (3). The two types of data were assembled independently and then matched to discover correlations. It was found that

in respect to no other complication of diabetes was the relationship of control more definite or more striking than in respect to nephropathy. There were 11 patients among the 451 who were judged to have had excellent control; none of these showed nephropathy. There were 50 other patients judged to have had good control; only 1 of these showed nephropathy. This was true despite the fact that of these 61 patients (11 with excellent and 50 with good control), 44 had had diabetes for twenty years or more. In contrast, 17 per cent of 92 patients with fair control, and 28 per cent of 298 patients with poor control gave evidence of serious renal disease.

This experience with diabetic nephropathy lends further support to the growing conviction that the sequelae of long-term diabetes are related to the degree of control maintained over the years. Although many points of basic importance remain obscure, from a practical standpoint the physician is obligated to plan treatment and supervise management in such a way as to provide the best degree of control attainable. With methods now available, "ideal" control is rarely, if ever, possible. However, unless the goal is set high, the result secured is almost certain to be less satisfactory. The hazards of producing psychologic trauma in the patients by insistence on a careful regimen have been overemphasized. A happy, well-adjusted and useful life is entirely compatible with care in diabetic management.

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3. KEIDING, N. R.; ROOT, H. F., and MARBLE, A.: Importance of control of diabetes in prevention of vascular complications, *J.A.M.A.* **150:** 964–969, 1952.

Letters to the Editor

A USEFUL AGENT FOR ORAL ALKALI THERAPY*

To the Editor:

There are certain medical conditions in which the physician finds it desirable to administer alkali to his patient, and sometimes for long periods of time. The variability in response to alkali therapy among patients with seemingly normal renal function is well known. In order to achieve urinary alkalinity, one patient may require large doses; another will respond to much smaller doses. The usual compounds prescribed for this purpose are sodium bicarbonate and sodium citrate. When given in quantities adequate for the desired end and over prolonged periods, both these drugs may produce annoying gastrointestinal symptoms and their taste palls on the user's palate. During some studies on skeletal metabolism, a solution of sodium citrate, potassium citrate and citric acid,[1] prepared in a flavored base in our laboratory, appeared to be well tolerated by the gastrointestinal tract in relatively high dosage. It was, therefore, given to patients with recurrent uric-acid and cystine stones as an alkalinizing agent (1).

The solution is so made up that it contains equimolar parts of sodium citrate, potassium citrate and citric acid, and each milliliter of the mixture contains 1 millimol of sodium, 1 millimol of potassium and 1 millimol of citric acid.[2] Dosage is thus easy to regulate. It may be taken diluted with ice and water as a soft drink or in teaspoonful doses followed by water.

Some patients have now taken as much as 120 cc. per day (30 cc. after each meal and at bedtime) for more than eighteen months without distress. An occasional patient has experienced mild transient diarrhea, but there have been no complaints of heartburn or bloating. All the patients who previously used bicarbonate or simple sodium citrate have stated that the

* The observations reported in this letter were made during a study of skeletal metabolism, under contract between the Atomic Energy Commission and the Johns Hopkins University.

[1] This combination of salts was suggested by Dr. H. E. Harrison, of the Department of Pediatrics, who was using this mixture in studying problems of calcium metabolism.

[2] Albright and associates used a somewhat similar mixture to the one described in this report in the treatment of a patient with renal acidosis, nephrocalcinosis and rickets, on the recommendation of Drs. A. M. Butler and A. T. Shohl (2). This mixture, however, contained only 1 mEq. of Na per ml.

mixture was far more pleasant to take, and that it was simpler to calculate the dose. Most of the 18 patients who have taken this medication have been able to maintain a urinary pH greater than 7.4 around the clock, without the necessity of waking and taking a 2 A.M. dose. Perhaps this is due to the fact that, with this mixture, larger doses of alkali may be given than could be tolerated if sodium citrate or bicarbonate alone were used.

The concentration of the mixture is such that it may readily be carried to work in the pocket, and dosage is easy to measure. Moreover, the solution is stable. Samples prepared over a year ago and standing at room temperature have shown no changes in pH (the pH is 4.8), no bacterial growth, no discoloration, no precipitation and no crystallization from solution. Samples kept at 34° F. for two months have also shown no crystallization.

The material also has the advantage of being inexpensive. As with any other alkalinizing agent, caution must be used in patients whose renal mechanisms may be deficient, for fear of developing edema, hyperkaliemia or alkalosis. We are not aware of any virtue of the added citric acid, save for the improved palatability. Serum citric acid concentration has not been measured, but patients taking 120 cc. of the mixture per day (equivalent to 23 grams of citric acid) have been found to excrete 800–900 mg. of citric acid per day,[3] whereas on control days without medication there was an excretion of approximately 500 mg. of citric acid. A dosage of 20 cc. of the solution taken four times per day has generally maintained a urinary pH of 7.4–7.6 throughout most of the twenty-four hours, without unpleasant side effects. Occasional patients require 120 cc. per day to achieve this status; others may need only 60 cc. per day. This alkalinizing mixture,[4] which was heretofore prepared in our laboratory, contains in each fluid ounce (30 cc.):

Citric acid	30 grains (2.0 Gm.)
Sodium citrate	45 grains (3.0 Gm.)
Potassium citrate	50 grains (3.3 Gm.)

incorporated in a palatable, non-alcoholic syrup base. The sugar content of the mixture is approximately 2 grams per 5 milliliters of solution.

HARRY EISENBERG
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The Metabolic Division of the Department of Medicine, The Johns Hopkins University and Hospital, Baltimore, Maryland

[3] These studies were made for us by Dr. H. E. Harrison.

[4] This preparation is now being supplied by the Willen Drug Company, 18 North High Street, Baltimore 2, Maryland.

Addendum

In answer to a question by one of the Editorial Board reviewers concerning the reason for including such a large amount of potassium in the solution, we have the following comments:

1. Mixtures of sodium citrate and citric acid alone in a 2 mM sodium citrate:1 mM citric acid ratio proved to be very flat in taste and unpalatable; they were, therefore, unsuitable for long-term administration. It would seem that the addition of potassium citrate to the mixture (in the equimolar ratio outlined in the letter) makes this solution much more pleasant to the taste.

2. Gudzent (Peters and Van Slyke: Quantitative Clinical Chemistry. I. Interpretations. Baltimore, The Williams & Wilkins Company, 1946, pp. 949–956) has shown that potassium urate has nearly twice the solubility of sodium urate in a given quantity of water.

3. There seemed to be no disadvantage to the use of potassium in metabolically normal persons, since most of it is absorbed and excreted promptly. The usual precautions outlined in the letter should, of course, be observed.

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THE SPECIFICITY OF THE REACTION OF PHENYLHYDRAZINE WITH 17,21-DIHYDROXY-20-KETOSTEROIDS

To the Editor:

Marks and Leftin (1) have reported that potassium iodide, chloral hydrate, and paraldehyde interfere in the Reddy, Jenkins and Thorn (2) procedure for the determination of 17,21-dihydroxy-20-ketosteroids. They interpret their findings as a "note of caution on the lack of specificity of the Porter-Silber reaction" and do not mention that their use of butanol for extraction may introduce specificity limitations in the procedure.

It is well known that reaction with phenylhydrazine to yield colored compounds is not in itself specific for the 17, 20, 21 configuration of hydrocortisone. Sugars, ascorbic acid, and many other substances with aldehyde or ketone groups react frequently with the development of a yellow color. Therefore, as noted by Silber and Porter (3), the specificity of any procedure employing this reaction for steroid analyses also depends upon the solvent used for the extraction and other steps prior to color development. In their procedure (3) chloroform is used as the solvent because it not only extracts the steroid satisfactorily but in addition is relatively non-polar;

TABLE 1. SAMPLE IN 10 ML. OF WATER EXTRACTED WITH 25 ML. OF CHLOROFORM, WASHED WITH 2 ML. OF 0.1 N NaOH; 10-ML. ALIQUOTS TAKEN FOR ANALYSIS (3)

Compound	Amount (mg.)	Optical density at 410 mμ
Acetyl salicylic acid	10	0
Ascorbic acid	10	0.004
Butazolidin	10	0.003
Chloral hydrate	10	0.003
Chlor-trimeton maleate	10	0.004
Dextrose	10	0
Estrone	0.5	0.003
Evan's Blue (T1824)	10	−0.002
1-Methadon	10	0.005
Pentobarbital sodium	10	0.002
p-Aminobenzoic acid	10	0
Penicillin G sodium	10	0
Potassium iodide	10	0.003
Pyridoxine hydrochloride	10	0
Riboflavin	0.1	0.002
Streptomycin sulfate	10	0
Sulfanilamide	10	0.004
Aminopyrine	10	0.009
Diparcol	10	0.213
Estradiol	0.5	0.012
Menadione	10	0.087
Paraldehyde	0.1	0.094
Progesterone	0.5	0.024
Quinine hydrocyloride	10	0.173
Hydrocortisone	0.02	0.437

thus, many potentially interfering substances are excluded by its use in the initial extraction step.

Nevertheless, substances which are soluble in chloroform in the presence of water or alkali, or soluble in the reagent in the presence of chloroform, and which react with phenylhydrazine, can interfere in the procedure. The optical densities observed when a random set of drugs, vitamins, one dye, and one salt were subjected to the procedure (3) are listed in the table.

Of those which gave significant results, only quinine showed an absorption peak at 410 mμ, with 10 mg. of the hydrochloride, giving approximately the same optical density as 8 micrograms of hydrocortisone. Colchicine (100 micrograms), not included in the table, gave high readings in both the sample and the blank (0.85–0.9), so this too could interfere with hydrocortisone assays if present in sufficiently high concentration. Po-

tassium iodide and chloral hydrate did not interfere significantly. Of all the substances tested, paraldehyde interfered most. (Both paraldehyde and colchicine can be determined by modifications of the procedure—paraldehyde by extraction with carbon tetrachloride and reading at 320 mμ, colchicine by the same procedure used for hydrocortisone but omitting the phenylhydrazine from the reagent and reading at 370–390 mμ.)

A point which illustrates the specificity of the procedure in the absence of interfering medication is the finding that the urine of adrenalectomized dogs contains no materials which react to give absorption at 410 mμ, even after glucuronidase treatment.

In conclusion, if one wishes to apply the test to the urine or plasma of subjects receiving medication, it should first be determined whether the drug interferes in the procedure. If the medication interferes, one must either avoid its administration until the samples are taken for analyses, make a correction by calculation, or remove it from the sample before color development. The latter may be accomplished by washing the aqueous sample with carbon tetrachloride, petroleum ether or iso-octane, since these solvents do not extract hydrocortisone or tetrahydrocortisone.

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The Endocrine Society

THE 1955 ANNUAL MEETING

The Thirty-seventh Annual Meeting of The Endocrine Society will be held in the Chalfonte-Haddon Hall Hotel, Atlantic City, New Jersey, on Thursday, Friday and Saturday, June 2, 3 and 4, 1955.

Dr. Matthew Molitch, 705 Pacific Avenue, Atlantic City, New Jersey, is in charge of the local arrangements for the meeting.

All Scientific Sessions will be held in the Chalfonte-Haddon Hall Hotel. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The annual dinner is scheduled for Friday, June 3, at 7:30 p.m., preceded by cocktails at 6:30 p.m.

All members are urged to make hotel reservations immediately, as the hotels expect to be filled to capacity. Correspond directly with Chalfonte-Haddon Hall, advising time of arrival and departure. Make your reservations now and avoid disappointment.

PROGRAM

THURSDAY, JUNE 2, 1955

9:30 A.M. THYROID

Chairmen: R. W. Bates and F. R. Keating, Jr.

1. THYRO-PITUITARY RELATIONS IN CRETINISM AND HYPOTHYROIDISM. *A. M. DiGeorge, S. A. D'Angelo** and *K. E. Paschkis.**

2. EARLY PREDICTION OF SUCCESS OR FAILURE OF RADIOIODINE THERAPY OF HYPERTHYROIDISM. *A. Schultz* and *L. Zieve* (introduced by E. B. Flink).

3. A METHOD FOR IODIDE BALANCE STUDIES IN ANIMALS ON LOW IODIDE DIETS. *L. Van Middlesworth.**

4. THERAPEUTIC IMPLICATIONS OF THE AVIDITY OF SOLITARY THYROID NODULES FOR I^{131}. *M. Perlmutter** and *S. L. Slater.*

5. DEIODINATION OF THYROXINE TO TRIIODOTHYRONINE BY KIDNEY SLICES OF RATS WITH VARYING THYROID FUNCTION. *F. C. Larson, K. Tomita* and *E. C. Albright.**

6. METABOLISM OF TRIIODOTHYRONINE AND THYROXINE IN PLASMA, PITUITARY AND CNS OF THE RABBIT. *J. Gross,** *D. H. Ford** and *M. Posner.*

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* Member.

INTERMISSION

7. A Defect of Thyroidal Storage in Subacute (Giant-Cell) Thyroiditis. *S. H. Ingbar** and *N. Freinkel.**

8. Placental Transfer of Thyrotropic Hormone in the Rat. *M. Nikitovitch* and *E. Knobil.**

9. A Clinical Appraisal of Radioiodine Tests of Thyroid Function. *W. M. McConahey,** *C. A. Owen* and *F. R. Keating, Jr.**

10. A Study of Endemic Goiter. *M. Roche, F. De Venanzi,** *J. Vera, E. Coll* and *M. S. Berti.*

11. A Comparison of Iodine Depletion Methods for TSH Assay in the Chick. *R. W. Bates** and *J. Cornfield.*

2:00 P.M. HYPOTHALAMUS AND PITUITARY

Chairmen: O. H. Pearson and G. Sayers

12. Evidence of Separate Hypothalamic Centers Controlling Corticotropin and Thyrotropin Secretion by the Pituitary. *M. A. Greer** and *H. Erwin.*

13. Effect of Hypothalamic Lesions on Blood ACTH Levels and 17-Hydroxycorticosteroid Secretion Following Trauma in the Dog. *D. M. Hume* and *D. H. Nelson.**

14. Role of the Neurohypophysis in the Release of ACTH. *M. Saffran, A. V. Schally* and *B. G. Benfey* (introduced by R. A. Cleghorn).

15. Pitressin and Blood ACTH in Adrenalectomized Rats. *G. Sayers** and *R. Burks.*

16. Changes in Blood ACTH under Various Experimental Conditions Using the Cross-Circulation Technique. *A. Brodish* and *C. N. H. Long.**

17. Increase in Circulating Red Cell Volume of Hypophysectomized Rats Injected with Plasma from Pregnant Rats. *A. N. Contopoulos, D. C. Van Dyke, M. E. Simpson* and *H. M. Evans.**

18. Influence of Growth Hormone and Cortisol upon Blood Formation in the Hypophysectomized Rat. *A. S. Gordon** and *G. J. Fruhman.*

INTERMISSION

19. Analysis of the Polyuria Produced by Hypophysectomy in Man. *M. B. Lipsett, J. P. Maclean. M. C. Li, C. D. West, B. S. Ray* and *O. H. Pearson.**

20. The Nature of the Effect of Alkali on Intermedin. *M. S. Raben.**

21. Effect of Partial Hypophysectomy on Adrenocortical, Thyroid and Gonadal Function in the Dog. *W. F. Ganong** and *D. M. Hume.*

22. Effect of Growth Hormone on Plasma Urea, Urea Excretion, Renal Function and Fasting Blood Glucose. *H. Gershberg** and *J. Gasch.*

23. Carbohydrate Metabolism in Intact, Hypophysectomized and Partially Pancreatectomized Rhesus Monkeys: Influence of Growth Hormone and Cortisone. *E. Knobil** and *R. O. Greep.**

24. Method for Measurement of Growth and Replacement of Cells in Man. *J. B. Hamilton.**

FRIDAY, JUNE 3, 1955

9:00 A.M. REPRODUCTION AND SEX HORMONES

Chairmen: R. O. Greep and L. Wilkins

25. Chorionic Gonadotrophin Titers Using *Bufo americanus* and *Bufo marinus*. *E. H. Hon* and *J. McL. Morris* (introduced by C. L. Buxton).

26. Congenital Gonadal Dysgenesis, Ovarian Agenesis, Male Pseudohermaphroditism: The Relationship to Theories of Human Sex Differentiation. *M. M. Grumbach, J. J. Van Wyk** and *L. Wilkins.**

27. Urinary Excretion of Interstitial-Cell Stimulating Hormone (ICSH) by Women with Normal and Abnormal Menstrual Function. *J. W. McArthur,** *F. M. Ingersoll* and *J. Worcester.*

28. Function of the Postmenopausal Ovary: Urinary Estrogen Excretion and the Response to Administered FSH. *C. A. Paulsen,** *R. B. Leach,** *H. Sandberg* and *W. O. Maddock.**

29. Eunuchoidism with Negative Follicle-Stimulating Hormone in the Female. *M. P. Moldawer, F. Albright,** *P. H. Benedict,** *A. P. Forbes** and *P. H. Henneman.**

30. Prolactin as a Factor in Luteinizing Hormone Assay. *A. Segaloff,** *A. Flores* and *S. L. Steelman.*

INTERMISSION

31. Effect of Depot Relaxin on Pubic Ligament Growth in Mice. *B. Kliman* and *R. O. Greep.**

32. Fractionation of Relaxin by Chromatography and Counter-Current Distribution. *E. H. Frieden* and *M. W. Noall* (introduced by W. H. Fishman).

33. Comparison of Oral and Subcutaneous Administration in Anabolic and Androgenic Effectiveness of 19-Nortestosterone and Testosterone. *D. E. Holtkamp,** *A. E. Heming** and *L. F. Mansor.*

34. The Metabolic Effect of 19-Nortestosterone. *W. H. Baker, P. H. Henneman,** *B. Baggett,** *L. L. Engel,** *D. Tibbets* and *M. Brown.*

35. Effect of Castration and Various Steroids on Individual Muscles of the Guinea Pig. *C. D. Kochakian,** *C. Tillotson* and *E. Dougherty.*

1:30 P.M. ANNUAL BUSINESS MEETING OF THE ENDOCRINE SOCIETY

2:30 P.M. CORTISOL DERIVATIVES AND ALDOSTERONE

Chairmen: E. H. Venning and L. T. Samuels

36. Inhibition of ACTH Activity in the Rat after Administration of 9α-Halogenated Steroids. *W. B. Kessler, R. F. Varney* and *A. Borman* (introduced by J. H. Leathem).

37. The Suppression of Endogenous Adrenal Cortical Secretion with 9-Alpha-Fluorohydrocortisone. *A. Goldfien,** *J. Saint-Marc, P. M. Beigelman* and *W. F. Ganong.**

38. Comparative Effect of Metacortandracin, 9-A-Fluorohydrocortisone, and Hydrocortisone upon ACTH Secretion in Man. *H. S. Kupperman,** *M. H. G. Blatt* and *H. Wiesbader.*

39. Distribution of Radioactive Hydrocortisone (Compound F) and Radioactive Corticosterone (Compound B) in Human Subjects. *A. A. Sandberg, C. Migeon** and *L. T. Samuels.**

40. Studies with a New Series of Steroids: Metacortandracin and Metacortandralone. *M. M. Pechet* and *F. C. Bartter.**

INTERMISSION

41. Acute Renal Response to Aldosterone: Physiological Basis for a New Assay Method. *G. W. Liddle,* J. Cornfield, A. G. T. Casper* and *F. C. Bartter.**

42. Secretion of Aldosterone by Hypophysectomized Dogs. *G. L. Farrell, E. W. Rauschkolb* and *S. Koletsky* (introduced by G. Sayers).

43. Studies on the Excretion of the Sodium-Retaining Corticoid. *E. H. Venning,* J. C. Beck, I. Dyrenfurth* and *J. P. Giroud.*

44. Effect of Aldosterone and Other Steroids on Renal Functions and Water Intoxication. *A. A. Renzi,* M. Renzi, J. J. Chart* and *R. Gaunt.**

45. Studies on Aldosterone in Man, *R. H. Sheppard, W. I. Morse, A. E. Renold* and *G. W. Thorn.**

6:30 P.M. ANNUAL DINNER

SATURDAY, JUNE 4, 1955

9:30 A.M. ADRENALS

Chairmen: G. W. Thorn and J. W. Jailer

46. Exchangeable Body Sodium and Potassium Measurements in Hyper- and Hypoadrenocorticism. *W. L. Arons,* B. Nusimovich* and *R. L. Vanderlinde.*

47. The Excretion of Pregnane-3α, 17α, 21-triol-20-one (Tetrahydro S) in Normal and Pathological Urine. *J. C. Touchstone, H. Bulaschenko, E. M. Richardson* and *F. C. Dohan.**

48. Response of Plasma 17-Hydroxycorticosterone Levels to ACTH in Adrenocortical Disease and in Pregnancy. *E. Z. Wallace, N. P. Christy* and *J. W. Jailer.**

49. Studies of Conjugates of C-21 Steroids in Human Plasma. *A. M. Bongiovanni** and *W. R. Eberlein.**

50. Metabolic and Endocrinologic Consequences of Abrupt Cessation of Long-Term Cortisone Therapy in Rheumatoid Arthritis. *E. Calkins,* W. Bauer,* L. L. Engel** and *P. Carter.*

51. Studies of the Kinetics of the Adrenal Cortical Response to Corticotropin. *V. DiRaimondo* and *D. Island* (introduced by P. H. Forsham).

INTERMISSION

52. Species Variations in the Response of Serum Proteins, Lipids and Lipoproteins to Cortisone. *E. T. Bossak, C. I. Wang* and *D. Adlersberg.**

53. The Prophylactic Use of Cortisone for Surgery of the Pituitary. *P. Troen* and *E. H. Rynearson.**

54. The Effect of Estrogen and Androgen on Calcium Metabolism during Cortisone Administration. *P. H. Henneman,* J. W. Irwin, D. M. K. Wang* and *W. S. Burrage.*

55. Addison's Disease with Normal Basal Secretion of 17-Hydroxycorticoids. *N. A. Haydar, J. C. Laidlaw,* W. J. Reddy** and *G. W. Thorn.**

56. Effects of Cortisone and Insulin on Urinary Excretion of Radioactive Vitamin B_{12} in Rats. *J. Meites** and *Y. S. L. Feng.*

57. Aminopyrine Inhibition of the Plasma 17-Hydroxycorticosteroid Increase Produced by the Pyrogenic Reaction. *N. P. Christy, A. Donn,* and *E. Z. Wallace* (introduced by J. W. Jailer).

2:00 P.M. JOINT SESSION WITH THE AMERICAN DIABETES ASSOCIATION

Chairmen: H. B. Mulholland and A. T. Kenyon

58. Hypophysectomy in Severe Juvenile Diabetes. *L. W. Kinsell,** *L. Lawrence* and *R. D. Weyand.*

59. The Metabolism of C^{14}-Labeled Glucose in Normal and Diabetic Subjects. *M. Miller, R. A. Shipley,** *N. Baker, W. W. Shreeve* and *J. W. Craig.*

60. Interactions of Growth Hormone, Cortisone, and Triiodothyronine in Experimental Diabetes in the Rat. *J. A. Owen, Jr., M. D. Bogdonoff* and *F. L. Engel.**

INTERMISSION

3:10 P.M. Banting Memorial Lecture

Influence of Epinephrine and Glucagon on Enzyme Systems in Liver and Muscle.

Professor Carl F. Cori, Washington University, St. Louis.

4:10 P.M.

61. Effects of Glucose Levels on Lipogenesis and Oxidation in Rat Liver and Adipose Tissue. *S. W. Milstein* and *F. X. Hausberger.**

62. The Distribution and Degradation of I^{131}-Labeled Insulin in Normals and Diabetics. *G. W. Welsh, E. D. Henley, R. H. Williams** and *N. J. Elgee.*

READ BY TITLE

Abstracts of papers read by title (Nos. 63–148) will be published along with the abstracts of the papers on the program, in the July issue.

THE 1955 AWARDS AND FELLOWSHIPS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship was established in 1947. It is designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. The Fellowship is awarded on alternate years (1955, 1957, etc.) and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst, McKenna and Harrison Fellowship may be made by any member of the Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than November 1 each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by November 1 each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

THE SEVENTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY AND THE INDIANA UNIVERSITY SCHOOL OF MEDICINE AT THE CONTINUATION STUDY CENTER OF THE INDIANA UNIVERSITY MEDICAL CENTER, INDIANAPOLIS, INDIANA

September 26–October 1, 1955

The faculty will consist of twenty-one eminent clinicians and investigators from various parts of the country in the fields of endocrinology and metabolism. The program will cover the various endocrinopathies, with emphasis on the clinical aspects, demonstration of laboratory tests, presentations of cases, and question-and-answer panel discussions. The course is designed to cover the main aspects of diagnosis and therapy in the field of endocrinology and metabolism for the physician in general practice and for those in other specialties who wish to have a general knowledge of this rapidly growing field.

A syllabus with brief abstracts of lectures will be available to the registrants at the time of the assembly.

For further information concerning the program, write Post Graduate Office, Indiana University Medical Center, 1100 West Michigan Street, Indianapolis 7, Indiana. Registration is limited to 100; tuition fee is $100.00. Arrangements for housing accommodations will be made through the Indiana Medical Center.

The American Goiter Association

The next annual meeting of the American Goiter Association will be held in Oklahoma City, Oklahoma, at the Skirvin Hotel, April 28, 29 and 30, 1955.

PRELIMINARY PROGRAM

Thursday, April 28, 1955—8:30 a.m.

1. Effect of Thyroid Activity on Brain Function and Brain Electrolyte Distribution in Rats.
   Paola S. Timiras, M.D., Ph.D. and *Dixon M. Woodbury, Ph.D.*, Salt Lake City, Utah.
2. Alteration of Thyroid Function Following Hypophysectomy in Man.
   M. C. Li, M.D., J. P. MacLean, M.D., M. B. Lipsett, M.D., J. E. Rall, M.D., B. S. Ray, M.D. and *O. H. Pearson, M.D.*, New York, N. Y.
3. The Histology of the Human Adenohypophysis in Some Thyroid Disorders, with Notes on the Effects of Therapy.
   Agnes Burt Russfield, M.D. Ph.D., Boston, Massachusetts.
4. Effect of Desiccated Thymus on the Potency of TSH and ACTH in the Chick.
   F. X. Gassner, Ph.D. and *M. L. Hopwood, M.S.*, Fort Collins, Colorado.
5. Role of Hexosamine-Containing Compounds in the Production of Experimental Exophthalmos.
   Norman F. Boas, M.D., Norwalk, Connecticut.
6. Studies on the Thyroidal Accumulation of Rhenium in the Rat.
   C. J. Shellabarger, Ph.D., Upton, L. I., New York.
7. A Study of the Trace Elements in the Thyroid in Normal and Pathological States.
   Henry J. Koch, Jr., M.D. and *Elmen R. Smith, M.A.*, New York, N. Y.
8. Serum Protein-Bound Iodine following Thyroxine in Cirrhosis, Diabetes, Pregnancy, Muscular Dystrophy, Neoplasia and Other Diseases.
   T. S. Danowski, M.D., R. Schwartz, M.D., G. J. Rhodes, M.D., R. D. Yoder, M.D., A. V. Puntereri, M.D. and *F. M. Mateer, M.D.*, Pittsburgh, Pennsylvania.

Thursday, April 28, 1955—2:00 p.m.

9. The Optimum Amount of Tissue to be Preserved at Subtotal Thyroidectomy (an Estimate Based on Serial Isotope-Tracer Measurements).
   D. Emerick Szilagyi, M.D. and *John L. Barrett, M.D.*, Detroit, Michigan.
10. The Treatment of Toxic Adenomatous Goiter by Large Doses of Radioactive Iodine.
    James R. Cook, M.D., Robert W. Jones, M.D. and *E. Perry McCullagh, M.D.*, Cleveland, Ohio.
11. The Use of Radioactive Iodine to Prepare Patients with Toxic Nodular Goiter for Surgery.
    K. R. Crispell, M.D., William Parson, M.D., Guy F. Hollifield, M.D. and *E. Meredith Alrich, M.D.*, Charlottesville, Virginia.

12. Results of Radioiodine Treatment of Euthyroid Patients with Angina Pectoris or Congestive Failure.
Henry L. Jaffe, M.D., Maurice H. Rosenfeld, M.D., Frederick W. Pobits, M.D. and *Laurence J. Stuppy, M.D.*, Los Angeles, California.
13. The Specificity of Thyroxine Binding by Serum Alpha Globulin.
Edwin C. Albright, M.D. and *Frank C. Larson, M.D.*, Madison, Wisconsin.
14. The Relation of Structure to *in vivo* and *in vitro* Activity of Thyroid Hormones.
Henry Lardy, Ph.D., Madison, Wisconsin.
15. The Mechanism of Action of the Antithyroid Action of Iodide Ion.
G. S. Serif, M.S. and *S. Kirkwood, Ph.D.*, Hamilton, Ontario, Canada.
16. The Mechanism of Action of Some Antithyroid Compounds.
L. C. Reid, M.D., New York, N. Y.

Friday, April 29, 1955—8:30 a.m.

17. Presidential Address
18. Van Meter Prize Essay
19. The Metabolism of Iodine in Cerebrospinal Fluid.
J. B. Alpers, M.D. and *J. E. Rall, M.D.*, New York, N. Y.
20. The Influence of the Thyroid on Adrenal Cortical Function.
Marvin E. Levin, M.D. and *William H. Daughaday, M.D.*, St. Louis, Missouri.
21. Triiodothyronine in the Serum of Patients Treated with Radioactive Iodine.
Richard S. Benua, M.D., Brown M. Dobyns, M.D., Ph.D. and *Anne Ninmer*, Cleveland, Ohio.
22. Nature of the I^{131} Compounds Appearing in the Thyroid Vein of Horse and Sheep after Injection of Iodide I^{131}.
Alvin Taurog, Ph.D., John D. Wheat, D.V.M. and *I. L. Chaikoff, M.D., Ph.D.*, Berkeley, California.
23. Diagnostic Accuracy of the Serum Protein-Bound Iodide.
Charles M. Blackburn, M.D. and *M. H. Power, Ph.D.*, Rochester, Minnesota.

Friday, April 29, 1955—2:00 p.m.

24. Childhood Thyroid Carcinoma: Pathologic Considerations and Their Therapeutic Implications.
J. A. Buckwalter, M.D., Iowa City, Iowa.
25. Thyroid Carcinoma in Children.
Theodore Winship, M.D. and *William W. Chase, M.D.*, Washington, D.C.
26. The Results of Conservative Operations for Low-Grade Carcinoma of the Thyroid.
George Crile, Jr., M.D. and *Beach Hazard, M.D.*, Cleveland, Ohio.
27. A Critical Evaluation of Surgical Treatment of Papillary Adenocarcinoma of the Thyroid. A Review of 200 Cases Followed from Five to Twenty-Five Years.
B. Marden Black, M.D. and *Mark L. Lemmon, M.D.*, Rochester, Minnesota.
28. The Pathological Evaluation of Radical Neck Dissection for Carcinoma of the Thyroid Gland.
William A. Meissner, M.D., Bentley P. Colcock, M.D. and *Hart Achenbach, M.D.*, Boston, Massachusetts.
29. Further Observations on the Treatment of Patients with Cancer of the Thyroid.
F. Maloof, M.D., A. Vickery, M.D. and *B. A. Rapp*, Boston, Massachusetts.

30. Gross and Microscopic Findings in Thyroid Glands from Patients with Clinically Normal Thyroids Glands.
J. D. Mortensen, M.D., Lewis B. Woolner, M.D. and *Warren A. Bennett, M.D.*, Rochester, Minnesota.

Saturday, April 30, 1955—8:30 a.m.

31. Factors Governing the Development of the Chick Embryo Thyroid. III—Histochemical Studies.
B. Dennis, M.D., Paul Wade, M.A. and *J. B. Trunnell, M.D.*, Houston, Texas.
32. Antigoitrogenic Effect of Casein.
L. Van Middlesworth, M.D., Ph.D., Memphis, Tennessee.
33. Hyperthyroidism in Children.
John C. McClintock, M.D., Thomas F. Frawley, M.D. and *John H. P. Holden, M.D.*, Albany, N. Y.
34. Studies in Non-Myxedematous Hypometabolism. I. The Clinical Syndrome and the Effects of Triiodothyronine or Combinations of Triiodothyronine and Thyroxine.
George S. Kurland, M.D., Milton W. Hamolsky, M.D. and *A. Stone Freedberg, M.D.*, Boston, Massachusetts.
35. Inter-Relationship of Hyperthyroidism and Thyroiditis.
Elmer C. Bartels, M.D., Boston, Massachusetts.
36. Malignant Lymphoma of the Thyroid Gland and its Relation to Hashimoto Disease. A Clinical and Pathologic Study of Eight Patients.
Stuart Lindsay, M.D. and *Morris E. Dailey, M.D.*, San Francisco, California.
37. Struma Lymphomatosa: Idiopathic Primary Compensated and Decompensated Thyroid Failure with Goiter.
Penn G. Skillern, M.D., George Crile, Jr., M.D., E. Perry McCullagh, M.D., John E. Hazard, M.D., Helen Brown, Ph.D. and *Lena A. Lewis, Ph.D.*, Cleveland, Ohio.
38. The Varied Symptomatology of Hypothyroidism.
H. Lisser, M.D., San Francisco, California.
39. Goiter and Hypothyroidism following Prolonged Administration of Iodide. *Henry H. Turner, M.D.* and *Robert B. Howard, M.D.*, Oklahoma City, Oklahoma.

Programs with abstracts will be sold at the meeting.

Events for wives of members and guests Thursday and Friday mornings.

AMERICAN MEDICAL WRITERS' ASSOCIATION

The 1955 meeting of the American Medical Writers' Association will be held in the Jefferson Hotel, St. Louis, Missouri, September 30–October 1. A special feature of the meeting this year will be the second half-day program (Saturday morning, Oct. 1) comprising a four-hour Workshop on Medical Writing to be conducted by faculty members from the Schools of Journalism of the Universities of Illinois, Missouri and Oklahoma. These universities are conducting four-year courses in medical journalism and medical writing, initiated and approved by this Association. Association members may attend this Workshop without charge; a $5.00 registration fee will be charged non-members.

In the January 1955 issue of the Mississippi Valley Medical Journal there is interesting information concerning the American Medical Writers' Association. The issue is devoted to papers read at the 1954 meeting in Chicago. These papers are available in a reprint entitled "1954 Symposia on Medical Writing" and may be secured for 25 cents postpaid from the Headquarters of the Association, 209–224 W. C. U. Building, Quincy, Illinois.

Harold Swanberg, M.D.
Secretary

THIRD CONGRESS OF FRENCH-SPEAKING ENDOCRINOLOGISTS

The meeting will take place in Brussels, Belgium, on June 18, 19 and 20, 1955.

The morning sessions will be devoted to the reading of reports concerning 1) hormonal balance in pregnancy, 2) androgens in the female, and 3) certain problems of cortisone therapy. The afternoon sessions will be devoted to communications relating to the problems raised by the reports.

Registration: Applications may be forwarded to *Professor J. Lederer, Secretary to the Congress. 233, avenue de Tervueren, Brussels, Belgium.*

The registration fee is fixed at 800 Belgian francs or $16, payable in Belgian francs at the "compte chèque-postal n° 624.794 de la Société belge d'Endocrinologie, compte Congrès" or in foreign currency by checks addressed to Professor Lederer.

THE 1955 LAURENTIAN HORMONE CONFERENCE

The Laurentian Hormone Conference of the AAAS will hold its 1955 annual meeting at the Hotel Stanley, Estes Park, Colorado, during the period September 11–16. Interested investigators and specialists in the hormone field may apply for attendance by writing to the Committee on Arrangements, 222 Maple Avenue, Shrewsbury, Massachusetts, for application blanks. Since accommodations at the hotel necessarily limit the attendance, only those persons submitting applications can be considered. The application blanks must be received by the Committee no later than May 6 in order to insure issuance of invitations as soon as possible thereafter.

The following program has been arranged:

I. HORMONE BIOSYNTHESIS AND METABOLISM

The Biosynthesis and Metabolism of Thyroid Hormone.
J. Roche, College de France

The Adrenal Medulla and the Formation of Pressor Amines.
Paul Hagen and A. D. Welch, Yale University Medical School

Biosynthesis of the Sterols and Steroid Hormones, with Particular Reference to the Estrogens.
R. D. H. Heard, McGill University School of Medicine

Biogenesis of Neutral Steroid Hormones.
Mika Hayano, N. Saba, R. I. Dorfman and O. Hechter, The Worcester Foundation for Experimental Biology

Enzymatic Mechanisms of Steroid Metabolism. (a) Oxidation-Reduction Reactions of the Steroid Nucleus. (b) Synthesis of Steroid Glucuronides.
K. J. Isselbacher and G. Tomkins, National Institutes of Health

II. HORMONES AND METABOLISM

Influence of Steroids on Cerebral Metabolism in Man.
G. S. Gordan, University of California Medical Center

Studies of Aldosterone in Relation to Water and Electrolyte Balance.
J. A. Luetscher, Jr., Stanford University School of Medicine

Tissue Metabolism and Growth.
M. E. Krahl, The University of Chicago

III. PITUITARY HORMONES

Human Urinary Gonadotrophin.
A. Albert, Mayo Clinic

Hormonal Control of Melanin Pigmentation.
A. B. Lerner, University of Oregon Medical School

Pituitary Syndromes in Man.
R. F. Escamilla, San Francisco, California

IV. SEX HORMONES

Male Sex Hormone and Its Role in Reproduction.
Thaddeus Mann, University of Cambridge, England

Clinical Studies of Testicular Hormone Production.
W. O. Maddock, C. A. Paulsen, and R. B. Leach, Wayne University College of Medicine

The Mechanism of Progesterone Effect on Uterine Muscle.
A. Csapo, Carnegie Institution of Washington

BOOKS RECEIVED

Acta Endocrinologica. Vol. XVII, Fasc. I–IV. Issue dedicated to Professor Axel Westman on the occasion of his sixtieth birthday. Fifty articles on endocrinological subjects; 460 pages; illustrated; 1954. Ejnar Munksgaard, Copenhagen, Denmark.

Analytical Cytology. Newer Methods for Studying Cellular Form and Function. Edited by ROBERT C. MELLORS, M.D., Ph.D., Sloan Kettering Institute for Cancer Research, Memorial Center for Cancer and Allied Diseases, Cornell University Medical College, New York, N. Y. Foreword by FRANCIS O. SCHMITT, Ph.D. Nine chapters, written by authorities in their respective fields, covering Methods in the Optical Spectral Region and Methods Using Electrons, Radioactive Isotopes, and X-rays; 522 pages; 144 illustrations; extensive bibliographies; 1955. McGraw-Hill Book Company, Inc., New York, N. Y. Price $15.00.

Biogenesi e Semeiologia Ginecologica del Pregnandiolo. By GUISEPPE VALLE E GERMANO FERRARIS, Clinica Ostetrica e Ginecologica dell'Università di Perugia; 152 pages; 30 illustrations; 1954. Il Pensiero Scientifico, Rome, Italy.

Endocrinologie du Médecin Praticien. By M. ALBEAUX-FERNET, Médecin des Hôpitaux de Paris; 37 chapters on endocrinological subjects; section on the therapeutic use of hormones; illustrated; 627 pages; 1955. Masson et Cie, Paris.

Examen Clinique et Traitement d'un Endocrinien. Travail de la Consultation d'Endocrinologie de L'Hôpital Lariboisière (Service du Professeur Agrégé S. de Sèze). By A. LICHTWITZ and R. PARLIER. Volume I. General; Hypophysis; Thyroid; Ovary; 456 pages; 131 figures; 1954; price 5.200 fr. Volume II. Testis; Adrenal; 392 pages; 86 figures; 1954; price 4.800 fr. Expansion Scientifique Française, Paris.

Hormonal and Neurogenic Cardiovascular Disorders. Endocrine and Neuro-Endocrine Factors in Pathogenesis and Treatment. By WILHELM RAAB, M.D., Professor of Experimental Medicine and Head of the Cardiovascular Research Unit, University of Vermont; 731 pages; 21 tables; 86 figures; 3,726 references; 1953. The Williams & Wilkins Company, Baltimore, Md. Price $15.00.

Potassium Metabolism in Health and Disease. By HOWARD L. HOLLEY, M.D., Department of Medicine, and WARNER W. CARLSON, Ph.D., Department of Biochemistry, University of Alabama Medical-Dental Schools, Birmingham, Alabama; Modern Medical Monograph No. 12; 144 pages; 11 illustrations; 1955. Grune & Stratton, Inc., New York and London. Price $4.50.

Refraction and Body-Growth. By G. S. PENDSE, Ophthalmic Surgeon, Poona, India. Published under the Authority and for The Indian Council of Medical Research, New Delhi, India, as Indian Medical Research Memoir, No. 38; 102 pages; 29 tables; 9 graphs; 3 figures; 1954.

Transactions of the Josiah Macy, Jr. Foundation Conferences. Publications Sales Office, P. O. Box 575, Packanack Lake, New Jersey.

Adrenal Cortex. Fifth Conference, November 4, 5 and 6, 1953, Princeton, N. J. Edited by ELAINE P. RALLI, M.D., Associate Professor of Medicine, New York University College of Medicine, New York; 187 pages; 7 tables; 27 figures; 1954. Price $3.75.

Connective Tissues. Fifth Conference, February 8, 9 and 10, 1954. Princeton, N. J. Edited by CHARLES RAGAN, M.D., Associate Professor of Medicine, Columbia University College of Physicians and Surgeons, New York; 222 pages; 6 tables; 55 figures (8 in color); 1954. Price $4.25.